1992

Assessment of mineral nutrition of declining forest trees with red spruce seedlings and indicator plants /

Bärbel Hölldampf
University of Massachusetts Amherst

ASSESSMENT OF MINERAL NUTRITION OF DECLINING FOREST TREES WITH RED SPRUCE SEEDLINGS AND INDICATOR PLANTS

A Thesis Presented

by

BÄRBEL HÖLLDAMPF

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

May 1992

Department of Plant and Soil Sciences

ASSESSMENT OF MINERAL NUTRITION OF DECLINING FOREST TREES WITH RED SPRUCE SEEDLINGS AND INDICATOR PLANTS

A Thesis Presented

by

BÄRBEL HÖLLDAMPF

Approved as to style and content by:

Allen V. Barker

Allen V. Barker, Chair

Matthew J. Kelty

Matthew J. Kelty, Member

Veneman

Petrus L. M. Veneman, Member

Lyle E. Craker

Lyle E. Craker, Department Head
Plant and Soil Sciences

To

Hans-Jürgen

ACKNOWLEDGMENTS

I am very grateful for the guidance and great encouragement which was given to me by Dr. Allen V. Barker. I thank Dr. Matthew J. Kelty and Dr. Petrus L.M. Veneman for sharing their knowledge.

I sincerely thank Clayton Carl for the red spruce seedlings and Dr. Gretchen Smith for the red spruce needle samples that I used in the study. I appreciate the friendly technical advice provided by Kathy M. Ready, Sarah A. Weis, Cynthia L. Barden, and Judith A. Bartos.

I thank my friends Polly, Deirdre and especially dear Cindy for all the fun we had together. I appreciated being welcomed as part of the department which made my studies in Amherst a very enjoyable experience.

ABSTRACT

ASSESSMENT OF MINERAL NUTRITION OF DECLINING FOREST TREES WITH RED SPRUCE SEEDLINGS AND INDICATOR PLANTS

MAY 1992

BÄRBEL HÖLLDAMPF, DIPLOM AGRAR INGENIEUR,

UNIVERSITÄT HOHENHEIM

M.S., UNIVERSITY OF MASSACHUSETTS

Directed by: Professor Allen V. Barker

Decline of high elevation forests in the northeastern United States has progressed in the last two decades. This phenomenon has been related to the impact of atmospheric pollution on forestal systems. The mineral nutrition of forest trees with abnormal growth appears to be affected. Declining forest trees showing chlorosis, needle loss, and dieback appear to be deficient in Ca and Mg. These deficiencies may be induced by nitrogenous nutrients borne in the atmosphere. Nitrogen may enhance growth of trees which leads to an exhaustion of Ca and Mg in the soil.

This study assessed mineral nutrition of healthy and declining red spruce (Picea rubens, Sarg.) and chlorotic balsam fir (Abies balsamea, L. Mill.) from sites in western Massachusetts. The effects of nitrogen and factors inherent in forest soil in mineral element accumulation by trees were evaluated with red spruce seedlings and radishes (Raphanus sativus, L.) in the greenhouse. Plants were grown in acid (pH < 4) O or A horizon from a Typic Haplorthod collected from a red spruce forest. Spruce or radishes were treated with a nutrient solution containing 7.5 to 17.5 mM NH_4^+ alone or 15 mM N of which NH_4^+ concentrations were 0, 3.75, 7.5, 11.25, or 15 mM with the remainder being NO_3^-.

Foliage of declining red spruce was low in P, Ca, Mg, and Mn relative to published standards. Chlorotic needles of balsam fir were low in K and Mg. Forest soils were apparently deficient in Ca and Mg. Radishes did not grow without $CaCO_3$ supplied to the forest soil. Liming and fertilization with calcium and magnesium salts increased dry weights, Ca, and Mg concentrations in leaves of radish. Without supplementary Mg, NH_4^+ was toxic to radishes. This toxicity occurred also if Ca supply was increased in the presence of Mg.

After 100 days of treatment with nutrient solutions, the spruce needles became chlorotic with 11.25 and 15 mM NH_4^+. Radishes exhibited NH_4^+ toxicity symptoms after 28 days. Plants had higher dry weights when grown in organic horizons than in mineral horizons. Plants grown in mineral horizons had higher N, K, and Ca but lower Mg and Mn concentrations in the leaves and needles. As NH_4^+ increased, plants had lower dry weights and less Ca accumulation. No other element (N, K, Mg, Mn) was affected by NH_4^+.

Mineral element deficiencies (P, K, Ca, and Mg) involved in the decline of trees at the studied sites appeared to be related to low elemental concentrations in the soil. The results of the greenhouse experiments show that NH_4^+ is a potential factor contributing to Ca and Mg deficiencies in forest trees. Calcium uptake was restricted by NH_4^+. Plants nourished with ammonium-N showed an increased requirement for Mg.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION AND OBJECTIVES

A. Introduction

The extensive forest decline that appeared in industrial countries in the early 1980's has been related to wet and dry deposition of atmospheric pollutants. The input of photooxidants, acids, and nitrogenous and sulfuric compounds in forest ecosystems has resulted in this multifactorial stress syndrome of decline (Tesche, 1991). In the northeastern United States, highly acid rain and fog (pH 2.8-4.5) in high-elevation forests are considered to be the cause of dieback in red spruce (*Picea rubens* Sarg.) and balsam fir (*Abies balsamea* L.Mill.) (Johnson et al., 1989; Bruck, 1985). The long term impact of increasing acidity and airborne contaminants in rain and fog caused a direct injury on the plant canopies and has altered soil chemistry, inducing changes in availability of mineral nutrient (Ulrich, 1990; Van Breemen et al., 1984). In acidified soils, cations, mainly calcium and magnesium, are exchanged by hydrogen and leached out. Aluminum and manganese are highly mobilized in soils below pH 4.2, and aluminum can become toxic to plant roots (Foy, 1984). Trees grown in calcium- and magnesium-depleted soils may become deficient in these elements.

A symptom indicating calcium deficiency is dieback of the stem tips or branches giving a flat-topped appearance to coniferous trees. Yellowing of older needles in conifers can be attributed to magnesium deficiency. In Germany, a decline symptom in the older needles of Norway spruce, described as "acute yellowing" typically starting at the needle tips and proceeded to the base of the needle. This was identified as magnesium deficiency (Kandler et al., 1987). In the forests of the northeastern United States, however, pronounced needle chlorosis is observed rarely before shedding of needles occurs (Bruck, 1985), indicating that magnesium deficiency may not be severe in this region.

Acidification and nutrient leaching from the soil does not fully explain decline in forests. Red spruce and Norway spruce as coniferous trees are known to acidify their environment due to their litter and are adapted to low base saturated soils derived from granite, gneiss, and schist that are low in nutrients (Riha et al., 1986). In search for a factor that could cause alterations in mineral nutrition of forest stands, the "Ammonium Hypothesis" (Nihlgard, 1985) has received much attention.

Nitrogen is an important growth limiting factor in forest ecosystems. Annual nitrogen mineralization in a mixed conifer stand in Maine (Federer, 1983) was between 2 to 6 kg N ha^{-1} on the forest floor and in the mineral soil. Nitrogen fixation by blue-green algae and lichens in a Massachusetts forest was less than 200 g ha^{-1} $year^{-1}$ (Tjepkema, 1979). Nitrogenous compounds in precipitation are nitric acid, nitrous oxides, and ammonium sulfate. Nitrous oxides originating from burning fossil fuels contribute 30% of total man-made nitrogenous air pollutants

(Tjepkema and Cartica, 1981) and add significant amounts of nitrogen into forests. Likens et al. (1977) recorded 22.9 kg nitrogen ha^{-1} yr^{-1}, 2.9 kg as NH_4^+ and 20.0 kg as NO_3^- in a New Hampshire forest. In another study in New Hampshire by Lovett et al. (1982), annual input was calculated to be 25 kg NO_3^--N ha^{-1} and 21 kg NH_4-N ha^{-1}. In Massachusetts, emissions from livestock manure and sewage sludge have been identified to be the source of 70% of the atmospheric ammonia accumulating an annual NH_4-N deposition rate of 2.5 kg ha^{-1} in a pine forest (Tjepkema et al., 1981). Additional available nitrogen to forest trees in general is beneficial to forest growth because forests are nitrogen deficient (Leyton, 1958). However, in case of deficiencies of other mineral elements (Ca, Mg, K, and P), additional nitrogen may cause cation-anion imbalances and influence physiological processes that contribute to the decline of conifers.

The impacts of nitrogen nutrition have been studied thoroughly in agricultural crops (Mulder, 1956), but few approaches have assessed the roles of nitrate and ammonia in forest nutrition. Perhaps agricultural crops for which the mineral nutrition has been well studied may be valuable for use in studies of the nutrition of forest trees. Barker et al. (1983) observed a strong inhibition of Ca and Mg uptake in radish (Raphanus sativus L.), when ammonium rather than nitrate was the nitrogen source. The fast-growing radishes that accumulate large amounts of mineral elements compared to the very slow-growing coniferous species may demonstrate nutritional imbalances due to ammonium nutrition in a relatively short time. Radishes will be used in this study to verify and monitor mineral element nutrition changes due to the treatments.

B. Objectives

1. To assess the soil characteristics and mineral nutrient composition of foliage of red spruce from provenances where forest decline is apparent, and
2. To determine the effects of ammonia and nitrate on foliar accumulation of mineral elements in red spruce seedlings and indicator plants.

CHAPTER II

LITERATURE REVIEW

Decline in red spruce has been observed at high elevations of the northeastern mountain ranges of the United States. Studies attributed the appearance of decline at 3000 to 4000 feet elevation to the fact that the summits are frequently submerged in the acidic fog of the clouds (Siccama et al., 1982, Scott et al., 1984). Since the 1960's, burning of fossil fuel and car traffic have increased concomitantly, releasing sulfur and nitrous oxides into the atmosphere. Nebe (1991) observed in a study of mineral element contents in spruce in the Thuringian Forest (former East Germany) from 1962 until 1984, an increase in nitrogen content and a 50 percent decrease in calcium and magnesium concentrations in presently declining trees. For trees fertilized with dolomite in 1960, calcium and magnesium deficiency were not present. In an earlier publication, Ilgen and Nebe (1989) reported an increase in nitrogen content starting as early as 1950 in a dendro-chronological analysis of the wood of spruce with needle yellowing.

Various studies with simulated acid rain demonstrated the capacity of tree foliage to absorb sulfuric and nitrogenous compounds. The foliar retention of ammonium-N is higher than that for nitrate-N as observed in analyses of throughfall and open-plot rain samples (Garten and Hanson, 1990). Simulated acidic fog (pH 5) destroyed the cuticular wax layer in Norway spruce (Picea abies Karst.) seedlings and caused yellowing, browning, and shedding of needles (Mengel et al., 1988). In soils, however, simulated acid rain increased the availability of Al and Mn and disturbed mineralization in the organic horizon (McColl and Firestone, 1987). MacDonald (1986) recorded decreased root to shoot ratios in seedlings of jack pine (Pinus banksiana Lamb.) with increasing soil acidity. The foliage N, K, Ca, Na, Zn, and Al concentrations increased as pH decreased whereas P and Mg concentrations in foliage fell. Schulze (1989) studied the nitrogen balance in a spruce forest in Germany. He detected preferential uptake of ammonia, leaching of nitrate and sulphate, and inhibition of cation uptake. As Mg, Ca, and K concentrations in the spruce foliage decreased, the P content and Al:Ca and Al:Mg ratios in the foliage increased. In a previous study Schulze et al. (1987) detected decreasing mycorrhizal growth on roots of Norway spruce with increasing NH_4^+ concentration in the soil solution. This decrease did not occur when the medium was less acidic and magnesium and calcium was added.

Huntington et al. (1990) showed that composition of foliage in red spruce is related to the cation content in the soil. They detected a positive relationship between foliar Ca and Mg concentrations of red spruce and cation content in the Oi and Oe horizon at Mount Moosilauke, New Hampshire. Friedland et al. (1988) measured low concentrations of P, Ca, Mg, and Mn in red spruce from high elevations in Vermont and New York. Simultaneously, protein-N was increased in samples of the foliage from high elevations. Browning due to chilling injury of the youngest needle year was noted, indicating decreased hardening capacity of the needles. In fertilization experiments with conifers, large amounts of nitrogen led to lessened wood growth and higher amino acid concentrations in the needles (Baule and Fricker, 1969).

In the Netherlands, where high ammonium sulfate concentrations in precipitation originating from intense agriculture are reported, deficiencies of K and Mg and premature shedding of needles in black pine (Pinus nigra var. maritima Ait.) have been observed (Roelofs, 1985). In healthy stands, the ammonia:magnesium ratio in soils was 6.5:1, whereas in soils from stands where black pine declined, the ratio was 22:1. Gijsman (1990b, c) studied the utilization of nitrate and ammonia at different soil acidities on Douglas-fir (Pseudotsuga menziesii Mirb.) seedlings. The uptake rates measured in terms of OH^- or H^+ excretion from the roots were in agreement with expected values. Plant growth was very poor when ammonia was the sole source of nitrogen. Those plants also had a low carbon:inorganic anion ratio due to the incorporation of ammonia in organic compounds. Edfast et al. (1990) compared spruce trees in declining forests with those in remote areas of Sweden where less air pollution exists. At sites with an annual input of 20 to 30 kg N/ha, free amino acid content in needles was increased. This observation was correlated with relatively low levels of K, Ca, and P in the foliage.

It can be concluded from the above mentioned studies that mineral nutrient deficiencies caused decline symptoms and that air-borne nitrogen, especially ammonium, may contribute to forest decline. Nitrate and ammonium absorbed by the canopy may enhance growth in the leaves that leads to physiological mineral element deficiencies due to low availability in the forest soil and slow translocation of these elements in the plant. Ammonium in the rhizosphere may inhibit the uptake of magnesium and calcium and lead to deficiencies in trees due to the competition for carriers in the root cell membranes. The present study was intended to investigate the effect of the nitrogen form on the mineral nutrition of red spruce seedlings in strongly acidic soil. Experiments with radishes that act as indicator plants were conducted. The radishes were grown in the same soils and subjected to the same experimental treatments as the red spruce seedlings. The experiments will help to test the hypothesis that Ca and Mg absorption in red spruce is inhibited by ammonia.

CHAPTER III

MINERAL ELEMENTS IN FOREST TREES AND SOILS

A. Introduction

Studies of forest decline compared trees and forest stands that appeared to be under different stages of decline (Friedland et al., 1988; Oren et al., 1988; LeBlanc, 1990; Edfast et al., 1990). Differences in wood growth, foliar composition, root morphology, and soil chemistry due to acid precipitation have been reviewed by Schulze (1989). Evidence has shown that mineral nutrient deficiencies were factors that contributed to forest decline (Kandler, 1987; Nebe, 1991). The impacts of air-borne nitrogen deposited on needles and forest floors that lead to nutritional imbalances were reviewed by Roelofs et al. (1985), Rollwagen and Zasoski (1988), and Gijsman (1990b).

The present study obtained information about foliar composition of red spruce from a healthy and a declining stand in western Massachusetts and needles from chlorotic fir trees at the summit of Mount Greylock. The objective was to search for indications of alterations in foliar composition of declining coniferous forest trees. Characteristics of a Spodosol in Mount Greylock State Reservation were evaluated to learn about possible impact of soil-borne factors on mineral nutrient concentrations in trees. The analysis focussed on the concentration of nitrogen, potassium, calcium, and manganese in foliage and in forest soils.

B. Materials and Methods

1. Mineral Analysis of Healthy and Declining Red Spruce

Leaf samples from mature red spruce stands (older than 50 years) in western Massachusetts were analyzed for N, P, K, Ca, Mg, and Mn contents. Three trees in a healthy stand (West Cummington, Hampshire Co., Mass.) and four trees in a declining stand (Becket, Berkshire Co., Mass) were rated in January 1989 by Brayton F. Wilson and Gretchen C. Smith from the Department for Forestry and Wildlife Management at the University of Massachusetts at Amherst. The trees at the Becket location were severely defoliated and had no needles older than nine years. From the upper crowns (upper 5-10 whorls) and the lower crowns (lower than 10 whorls from the top) of each tree, three branches were selected. Branches in the upper crowns were not older than 13 years, whereas in the lower crown branches were over 20 years old. From the three branches, samples of one-year-old, two-year-old, and five-year-old needles were detached, and needles of the same age were combined. Needles were combined using 5 grams from each of the three branches to obtain a homogeneous sample. Total nitrogen was determined by Kjeldahl digestion and distillation. Phosphorous was assessed colorimetrically (Watanabe and Olson, 1965). Potassium, calcium, magnesium, and manganese were determined by atomic absorption spectrophotometry of the dry ashed samples.

2. <u>Sampling and Analysis of Balsam Fir Needles</u>

Branches from four balsam fir (*Abies balsamea* (L.) Mill.) at the top of Mount Greylock, Massachusetts were collected on November 20, 1991. The trees had similar heights and appeared to be in the same state of discoloration. The one-year-old needles had a dark blue-green color. The two-year-old needles were green with some yellow tips. Chlorosis of the entire needles was observed in the three-year-old needles. Needles older than three years had brown speckles, and branches towards the inside of the crown were defoliated.

Three-year-old branches were taken from the mid-crown and the center of the limbs and stored frozen. One-, two-, and three-year-old needles were detached with a razor blade, separated, and used for chlorophyll a and b extraction in 80% acetone (Holden, 1965). The detached needles were dried, ground, and ashed. Mineral element concentrations (potassium, calcium, magnesium and manganese) were determined in the ash. Total nitrogen was determined according to the Kjeldahl method.

3. <u>Forest Soil Sampling and Analysis</u>

Soils from forest stands on the foot of the westfacing slope of Mount Williams at 2300 ft elevation in Mount Greylock State Reservation, Berkshire County, Massachusetts, were collected for mineral analysis. At this site, the red spruce exhibited decline symptoms (needle-loss and death of trees) and was in a mixed stand with hardwood species.

Soils in The Greylock Range developed on glacial till derived from schist and are classified as loamy, mixed, frigid Lithic Haplorthods of the Lyman series and coarse-loamy, mixed, frigid Typic Haplorthods of the Tunbridge series (Scanu, 1988).

Two soil horizon descriptions were made on October 20, 1991, in a oak, birch, red spruce stand and in a red spruce stand (Soil Survey Staff, 1990). Soil samples were collected from each horizon for mineral analysis. Two separate samples of 500 grams from the O and A horizons were collected on April 7, June 16, October 20, 1991, in an area of 10 meters radius around the center of the plots where the profile hole was dug later. From the air-dried soil samples, stones and coarse organic material were removed using a 2-mm mesh sieve. Concentrations of calcium, magnesium, and potassium were determined by extraction of soils in 1 M NH_4 acetate (Rich, 1965). Ammonium in soil was extracted by 1 M KCl solution (Bremner, 1965), and nitrate in soil was extracted in water and measured colorimetrically (Cataldo et al., 1975). Organic carbon was determined by ignition in a muffle furnace at 500°C. Soil pH was measured potentio-metrically in 1:1 soil water-suspension for the mineral soil and in 1:5 soil-water suspension for the organic soil.

4. Statistical Analysis

Red spruce stands were treated as individual plots the trees being replicates. Data were processed by analysis of variance (ANOVA). The forest site was the main treatment, position in the crown, and needle ages were the subplots. Means of mineral element concentrations of the stands and crown positions were compared by least significant differences (LSD). Regression analysis was employed to compare effects of needle ages. Data obtained from the balsam firs were processed by ANOVA, the individual trees were the replicates. Needles ages were the independent variable for the regression analysis. For the data obtained from the organic and mineral soil horizons, differences were determined by a paired t-test.

C. Results

1. Mineral Element Composition of Healthy and Declining Red Spruce

Elemental composition of trees in healthy and declining stands was determined for needles of different ages in the upper and lower crown positions (Table 3.1.).

The overall means for the stands of phosphorous, calcium, magnesium and manganese were significant (Table 3.2). Trees in the healthy stand had 0.3 mg P, 1.5 mg Ca, 0.2 Mg, and 0.9 mg Mn g^{-1} dry weight higher concentrations than those in the declining stand (Table 3.1). Differences between the stands for phosphorous and manganese were significant at $P \leq 0.05$ and those for calcium and magnesium were signifiant at $P \leq 0.1$ (Tables 3.1 and 3.2). Differences in nitrogen and potassium between the stands were not significant (Table 3.2).

Table 3.1. Mineral element concentrations in red spruce needles from a healthy and a declining forest stand.

	Elemental Concentrations (mg g^{-1} dry wt.)											
Age	Healthy Stand						Declining Stand					
(years)	N	P	K	Ca	Mg	Mn	N	P	K	Ca	Mg	Mn
							upper crown					
1	11	1.4	4.8	3.2	0.8	1.2	12	1.1	5.0	3.1	0.7	0.4
2	11	1.3	4.6	3.7	0.7	1.3	11	0.9	4.6	3.1	0.6	0.5
5	10	1.0	3.4	4.8	0.5	1.3	10	0.8	4.1	3.8	0.3	0.3
mean	11	1.2	4.2^{B}	3.9^{b}	0.7^{A}	1.2	11	0.9	4.5^{AB}	3.3^{c}	0.5^{B}	0.4
							lower crown					
1	10	1.4	5.5	4.1	0.9	1.1	11	1.1	4.7	2.9	0.7	0.4
2	11	1.3	5.3	5.7	0.8	1.4	12	0.9	4.7	2.8	0.6	0.4
5	9	1.0	4.2	7.2	0.5	1.6	10	0.8	3.0	3.9	0.4	0.3
mean	10	1.2	5.0^{A}	5.7^{a}	0.7^{A}	1.4	11	0.9	4.1^{B}	3.2^{c}	0.5^{B}	0.4
MEAN	11	1.2*	4.6	4.8†	0.7†	1.3*	11	0.9	4.3	3.3	0.5	0.4

Means for comparisons of crowns and sites are significant at $P \leq 0.1$ and $P \leq 0.05$ if followed by a different upper or lower case letter, respectively.
† $P \leq 0.1$, and * $P \leq 0.05$ for comparisons of stands.

Table 3.2. Results of ANOVA and regression analysis for needle age of elemental concentrations in red spruce.

Source	Mineral Elements					
	N	P	K	Ca	Mg	Mn
	Significance of the F-Value					
Stand (S)	0.474	0.022	0.542	0.070	0.066	0.023
Position (P)	0.435	0.770	0.523	0.243	0.075	0.472
Age (A)	0.000	0.000	0.000	0.001	0.000	0.366
S x P	0.730	0.194	0.014	0.000	0.954	0.326
S x A	0.772	0.003	0.988	0.029	0.097	0.224
P x A	0.527	0.834	0.482	0.049	0.287	0.203
S x P x A	0.670	0.777	0.440	0.122	0.266	0.138
	Significance of Regression against Age of Needles					
	Healthy Stand					
Linear	**	***	**	*	***	-
Quadratic	*	-	-	-	-	-
	Declining Stand					
Linear	**	***	**	**	***	-
Quadratic	-	-	-	*	*	-

*, **, and *** significant at $P \leq 0.05$, 0.01, and 0.001, respectively.
-, nonsignificant.

Crown position had little effect on mineral element concentrations in foliage of red spruce. Concentrations of potassium in the lower crowns of healthy trees were 0.8 mg g^{-1} higher than those in the upper crowns, whereas in the upper crowns of declining trees 0.4 mg g^{-1} higher potassiumconcentrations were present compared to those in the lower crowns (Table 3.1). Differences of potassium between crown positions were significant at $P \leq 0.1$ (Table 3.1).

The calcium concentrations in the lower crowns of trees in the healthy stand were 1.8 mg g^{-1} higher than those in the upper crowns (Table 3.1). Calcium concentrations in the lower crowns of healthy trees were 2.5 mg g^{-1} higher than those in the lower crowns of declining trees (Table 3.1). Differences among crown positions of healthy and declining trees were significant (Table 3.1). An accumulation of calcium in the lower crown of declining trees was not observed. Magnesium and manganese concentrations were not different for crown position within the trees for healthy or declining trees (Tables 3.1 and 3.2). Magnesium was significant for crown position at $P \leq 0.1$ between healthy and declining trees (Table 3.1).

In five-year-old needles nitrogen, phosphorous, potassium, and magnesium concentrations were lower and calcium concentrations higher than those in one-year-old needles (Table 3.1). The effect of age on elemental concentrations in needles was significant in both stands for nitrogen, phosphorous, potassium, and manganese (Table 3.2). The regression for the stands separately was significant for calcium and magnesium but not for manganese (Table 3.2.). In healthy and declining trees, nitrogen and phoshorous fell linearly between one- and five-year-old needles (Table 3.2).

Potassium between one- and five-year-old needles decreased by 1.3 and 1.7 mg g^{-1} in lower crowns of healthy and declining trees, respectively. In the upper crowns of healthy trees five-year-old needles had 1.4 mg K g^{-1} less than one-year-old needles, whereas the difference in declining trees was only 0.9 mg K g^{-1}. Potassium concentrations fell linearly with increasing age of needles (Tables 3.1 and 3.2).

Calcium concentrations in five-year-old needles of upper crowns in the healthy trees were 1.6 mg g^{-1} higher and on the lower crown 3.1 mg g^{-1} higher than those in one-year-old needles and increased linearly with needle age (Tables 3.1 and 3.2). Differences of calcium in one- and five-year-old needles in the foliage of declining trees was 0.7 mg g^{-1} in the upper crown and 1.0 mg g^{-1} in the lower crown (Table 3.1). The regression for calcium against age of needles was linear (Table 3.2).

Magnesium decreased between one- and five-year-old needles by 0.3 and 0.4 mg g^{-1} in upper and lower crowns of healthy and decling trees (Table 3.1). Magnesium concentrations decreased strongly linear with increased age of needles (Tables 3.1 and 3.2). For manganese no trend was significant regarding needle age (Table 3.2).

2. Mineral Element Composition and Chlorophyll Concentrations in Balsam Fir

Mineral nutrient and chlorophyll concentrations in chlorotic balsam fir trees from Mount Greylock were evaluated for one-, two-, and three-year-old needles (Table 3.3). Nitrogen, potassium, magnesium, and chlorophyll concentrations decreased as the age of the needles increased. The difference in nitrogen between one- and three-year-old needles was 3 mg g^{-1}. Potassium, magnesium, and chlorophyll concentrations in one-year-old needles were approximately 30 % less than those in three-year-old needles. Nitrogen, potassium, magnesium, and chlorophyll concentrations decreased linearly with leaf age. The regression was highly significant ($P \leq 0.001$) for magnesium and chlorophyll. Calcium and manganese concentrations were lowest in the youngest needles. Calcium concentrations were not different among the needle ages.

The trees which appeared similar in discoloration had variable concentrations of potassium (2 to 3.1 mg g^{-1}), calcium (1.6 to 9.3 mg g^{-1}), magnesium (0.5 to 0.7 mg g^{-1}), manganese (0.2 to 0.8 mg g^{-1}), and chlorophyll (0.5 to 1.0 mg g^{-1}) in one-year-old needles. Nitrogen did not vary among trees (12 to 14 mg g^{-1}).

Table 3.3. Concentrations of mineral elements and chlorophyll, results of ANOVA, and regression analysis of balsam fir needles from Mount Greylock.

Age	Elements (mg g^{-1})[x]					
(year)	N	K	Ca	Mg	Mn	Chl
1	13	2.7	4.0	0.6	0.3	0.7
2	12	2.1	5.1	0.5	0.4	0.5
3	10	1.9	4.8	0.4	0.4	0.5
	Results of ANOVA					
	Significance of F-value from ANOVA					
Tree (Rep)	0.451	0.045	0.002	0.000	0.000	0.000
Age	0.016	0.014	0.812	0.000	0.028	0.000
	Significance of Regression against Age of Needles[y]					
Linear	**	**	-	***	**	***
Quadr.	-	-	-	-	-	*

[x] Chemical elements mg g^{-1} dry weight. Chlorophyll mg g^{-1} fresh weight.
[y] * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

3. Forest Soil Acidity, Mineral Element and Organic Matter Concentrations

Two soil profiles that were dug on the westfacing slope of Mount Williams in Mount Greylock State Reservation exhibited common physical and chemical features (Table 3.4). Soil development reached to a depth of 60 cm to the greenish mica schist parent material. The texture was sandy loam with 5 % gravel by volume. At 40 cm depth in the BC horizon, a few, coarse, distinct low chroma (7.5 YR 7/2) and high chroma (7.5 YR 5/8) mottles indicated presence of a seasonal water table and a moderately well drained soil. The spodic (Bs) horizon in both profiles had a bright orange color (10 YR 5/6). The boundary to the A horizon was wavy and the A horizon material was mixed within the Bs horizon due to windthrow of trees.

In the red spruce stand, only needle litter contributed to the O horizon, and no undergrowth was present. The A horizon in the red spruce stand was very dark brown (7.5 YR 3/3) and was thicker than in the hardwood stand. The organic material in the hardwood stand consisted of litter from paper birch, oak, hemlock, ferns, and red spruce. The A horizon in the hardwood stand was dark brown (7.5 YR 4/4) and the Bs1 horizon was yellowish brown (10 YR 5/4). The profile was mapped as a Typic Haplorthod (Soil Survey Staff, 1990). In the O and A horizons in the hardwood and red spruce stand, fine roots were plentiful. Coarse roots of

0.5-cm diameter with fibrous laterals growing deeper than 60 cm.

Soil organic matter content decreased with depth (Table 3.4). Soil pH rose with depth. Concentrations of calcium, magnesium, and potassium were relatively high in the O horizon. Calcium was the most abundant cation in the O horizon. Mineral element concentrations fell abruptly from the organic to the mineral horizon. Not much difference in cation concentrations occurred between the mineral horizons.

According to a t-test, calcium, magnesium, and potassium concentrations in the organic horizon were significantly higher than those in the mineral horizon (Table 3.5). The soil samples taken in April contained somewhat higher concentrations of calcium, magnesium, and potassium than those taken in June and November (Table 3.5.). Calcium concentrations especially tended to decrease between April and November in the O and A horizons. Magnesium, potassium, and pH were less affected than calcium. Concentrations of 2.0 to 8.0 mg NH_4^+ kg^{-1} air-dried soil were measured in the organic soil. The mineral horizons had no detectable NH_4^+. If the weight of the organic horizon is estimated to be 250,000 kg (5.0 cm thickness, 0.5 kg dm^{-3}), the total amount of ammonium ha^{-1} would be 0.5 to 2.0 kg. Nitrate nitrogen in each horizon was below the detection range of 1.0 mg kg^{-1}.

Table 3.4. Acidity, organic matter and mineral element concentrations in two Typic Haplorthod profiles in Mount Greylock State Reservation, Massachusetts.

Horizon	depth (cm)	OM(%)[x]	pH	Element (meq kg^{-1})		
				Ca	Mg	K
			Hardwood-Red Spruce			
O	7-0	78	3.4	25.0	8.8	10.9
A	0-16	16	3.7	1.6	1.4	1.2
Bs	16-40	14	3.9	0.9	0.7	1.0
BC	> 40	2	4.4	1.3	0.3	1.4
			Red Spruce			
O	6-0	87	3.6	66.1	18.5	21.1
A	0-11	63	3.9	2.4	2.6	2.2
Bs	11-35	14	4.1	0.8	0.7	1.2
BC	35-60	7	4.2	0.6	0.4	0.3

[x] Organic matter, loss on ignition.

Table 3.5. Acidity and mineral element concentrations in soil samples from a hardwood-red spruce and a red spruce stand in Mount Greylock State Reservation, Massachusetts, collected on three different dates.

			Element meq kg^{-1}		
Horizon	Date	pH	Ca	Mg	K
			Hardwood-Red Spruce		
O	4/9/91	3.8	84*	20*	20*
	6/16/91	3.6	77*	14*	26*
	11/20/91	3.7	45*	14*	21*
A	4/9/91	3.9	5	2	2
	6/16/91	3.7	4	1	1
	11/20/91	3.7	2	1	1
			Red Spruce		
O	4/9/91	3.7	116*	19*	21*
	6/16/91	3.7	40*	13*	15*
	11/20/91	3.6	42*	9*	17*
A	4/9/91	3.8	6	2	2
	6/16/91	3.9	2	2	2
	11/20/91	3.6	3	1	1

* Elements in O horizon are different from A horizon by t-test ($P \leq 0.01$) by date.

D. Discussion

1. Red Spruce

The present study assessed the mineral element composition in a red spruce stand which appeared to be under decline compared with that of a healthy red spruce stand. The following discussion will evaluate as to whether the declining stand suffered mineral element deficiencies and if there were indications for the causes of any elemental disorder.

Studies in Germany have detected mineral nutrient deficiencies in Norway spruce [*Picea abies* (L.) Karst.] in which chlorotic needles and needle loss were apparent. Critical foliar concentrations for spruce were determined as guidelines for forest fertilization (Süchting, 1949; Wittich, 1959). Needles should not contain less than 12.0 mg N g^{-1}, 1.2 mg P g^{-1}, 4.0 mg K g^{-1}, 3.5 mg Ca g^{-1}, and 0.5 mg Mg g^{-1} to provide healthy tree growth and maximum wood growth. Recent studies reported that healthy spruce had more than 0.5 mg magnesium g^{-1} and 4.0 mg calcium g^{-1} dry weight in two-year-old needles (Zech and Popp, 1983; Kandler et al., 1987) and that chlorotic spruce had magnesium concentrations as low as 0.3 mg g^{-1} in one-year-old needles (Schulze et al., 1987). Zöttl (1990) suggested a requirement of higher than 12 mg N, 1.2 mg P, 4 mg K, 3.5 mg Ca, and 0.6 mg Mg g^{-1} dry weight in needles from top whorls in order to avoid mineral nutrient deficiencies in Norway spruce and to prevent restricted wood production.

Huntington et al. (1990) reported concentrations between 1.0 to 1.4 mg P g^{-1}, 1.9 to 3.2 mg Ca g^{-1} and 0.6 and 0.7 mg Mg g^{-1} in dry needles of apparently healthy red spruce at Mt. Moosilauke, New Hampshire.

As opposed to chlorosis as a symptom of decline in Norway spruce, red spruce in the northeastern United States seldomly exhibits needle yellowing (Bruck, 1985). Bruck (1985) also observed that trees in a declining stand lost large amounts of needles without showing chlorosis in the remaining foliage. The nitrogen and potassium concentrations in the foliage of the red spruce in the present study were not different between the healthy and the declining stand. Trees accumulated around 11 mg nitrogen g^{-1} and 5 mg potassium g^{-1} in one-year-old needles (Table 3.1.). These concentrations were low compared to those reported in the literature. The nitrogen and potassium concentrations of one-year-old needles of healthy Norway spruce given in the studies by Senser and Höpker (1989) were 15.0 and 7.0 mg g^{-1}, respectively. According to the standards reported by other workers (Süchting, 1949; Baule and Fricker, 1967), nitrogen and potassium therefore, appeared to be limiting in both the healthy and declining trees in the present study.

Healthy trees had higher phosphorous concentrations that were sufficient at 1.4 mg g^{-1} in one-year-old needles as opposed to levels in declining trees which were less than 1.2 mg g^{-1} and insufficient when compared to critical concentrations (Wittich, 1959; Zöttl, 1990). Calcium accumulated in the lower crowns and five-year-old needles of healthy trees. Calcium was expected to be higher in older tissue because it is transported by the transpiration stream in the xylem and not translocated from its original site of deposition due to its immobilization in the tissue. Declining trees in the present study had lower overall calcium and little accumulation of calcium in the older foliage. Calcium concentrations of 3.2 mg g^{-1} in one-year-old needles of the upper crown were critical according to the 3.5 mg g^{-1} threshold value obtained from Norway spruce but were normal compared to those reported by Huntington et al. (1990). Schulze et al. (1987) detected lower calcium accumulation in the older needles of declining Norway spruce and suggested an efflux of calcium due to foliar leaching.

Low acquisition of calcium in the declining trees may be inherent to the soil or due to the morphology of the root system. Huntington et al. (1990) found a positive correlation between the calcium and magnesium concentrations in the foliage of red spruce and the amount of exchangeable calcium and magnesium in the O horizon of a soil at Mount Moosilauke, New Hampshire. Schulze et al. (1987) observed a high abundancy of root tips and mycorrhizal infected fine roots in nutrient-rich O horizons. They also reported that the composition of the xylem sap in Norway spruce was correlated with the calcium and magnesium concentrations detected in the O horizon and in the foliage. The relationship between fine roots located in the O horizon and mineral nutrition was apparent during drought, for mineral element concentrations in the xylem sap decreased when the O horizon dried.

Becket soils, in the declining stand, are Typic Fragiorthods on granitic gneiss bedrock that are lower in calcium than the micaceous schist bedrock in the nondeclining stand at West Cummington. In the O horizon of the Becket site, concentrations of calcium, magnesium, and potassium were 25.0, 5.7, and 3.8 meq kg^{-1}, respectively (W.J. Manning, Univ. of Massachusetts personal communication). These calcium concentrations are somewhat lower than in the O-horizon of the high elevation hardwood forest (Table 3.4. and 3.5.) soils of which were derived from mica schist.

The manganese concentrations in the red spruce in the declining stand was 30 % of that in the healthy trees (Table 3.1.). Little is reported about manganese deficiency in coniferous trees grown in acid soils. Huntington et al. (1990) reported 0.7 to 1.3 mg Mn g^{-1} in young needles of healthy red spruce. In Norway spruce grown on calcareous bedrock, manganese deficiency was observed at needle concentrations of 0.004 to 0.015 mg g^{-1} (Zech, 1973). According to these figures, neither manganese toxicity nor manganese deficiency occurred in the red spruce from the sites in West Cummington and Becket.

Another aspect of the present study was to compare the mineral element concentrations in the upper and lower crowns of the trees. Calcium concentrations in the lower crowns of healthy trees were higher than those in the upper crown, an observation which was not true in the declining trees. This difference could be an indication for disturbed Ca nutrition in the declining trees. Phosphorous and magnesium concentrations were not different between the upper and the lower crown in the healthy or declining trees. These results suggested that phosphorous and magnesium were well distributed throughout the crown. If deficiency was present, it was not severe enough to suggest translocation of magnesium into the upper part of the crown with the resulting depletion of phosphorous and magnesium in the lower crown. There is little information in the literature about the distribution of mineral elements in crowns of coniferous trees. In most studies available, foliage samples were taken from branches in upper crown parts of forest trees, ignoring the lower canopy.

Differences in magnesium concentrations among needle ages suggest possibilities of magnesium deficiencies. In one-year-old needles of red spruce from the healthy site in West Cummington or the declining site in Becket (Table 3.1), magnesium concentrations were between 0.7 and 0.8 mg Mg dry weight and can be considered as sufficient based on the results of previous studies (Süchting, 1949; Wittich, 1959;

Zöttl, 1990). In five-year-old needles from the declining site, the magnesium concentrations were deficient ($0.3\ mg\ g^{-1}$).

Magnesium deficiency in older needles may not represent the health status of a tree, for magnesium is translocated from older needles to the younger needles. Schulze et al. (1987) estimated that $0.13\ mg\ g^{-1}$ of the $0.5\ mg\ g^{-1}$ magnesium in the dry one-year-old needles of Norway spruce originates from the older needles with the remainder being delivered by the stem directly from the soil. Older Norway spruce needles became chlorotic when the delivery of magnesium from the soil was inhibited. The relationship between magnesium and nitrogen in mature needles from Norway spruce was studied thoroughly by Schulze (1989). Magnesium was translocated into younger needles during the growing season. The rate of the translocation of magnesium was dependent on the nitrogen concentrations in one-year-old needles. The higher the nitrogen levels in the youngest leaves, the more advanced the magnesium deficiency of the older needles. Rapidly developing chlorosis in Norway spruce was observed in many locations in Germany and described as "acute yellowing" (Kandler et al., 1987). A magnesium fertilizer amendment allowed greening of formerly chlorotic needles (Kaupenjohann et al, 1987; Beyschlag et al., 1987).

Trees at the declining site which suffered severe needle loss differed little in nitrogen and potassium concentrations but exhibited lower phosphorous, calcium, and magnesium concentrations compared to trees at the healthy site. Nitrogen and potassium concentrations in the foliage of healthy and declining trees were at incipient deficiency according to values reported in the literature. Calcium was probably less abundant in the soil derived from granitic gneiss in the declining stand. It is most likely that trees at the healthy and the declining site were at the threshold of deficiency in plants nutrients and that in declining trees phosphorous, calcium, and magnesium in particular became limiting. The mineral nutrient deficiencies in the red spruce are probably soil inherent and may have originated from element depletion in the soil.

2. Balsam Fir

Mineral elements in balsam fir from Mount Greylock were determined to evaluate mineral nutrient deficiencies as the cause of the observed discoloration in the foliage. All mineral elements except nitrogen were highly variable among the trees. The chlorotic, three-year-old needles had relatively high nitrogen concentrations compared to the two-year-old, normal appearing of red spruce (Table 3.1.). Some of the nitrogen supplied to the plants may originate from the absorption of atmospheric nitrogen from clouds and mist. Firs were at a higher elevation than the red spruce. Lovett et al. (1982) reported a high efficiency of balsam fir forests to capture cloud droplets. Balsam firs at 3600 ft elevation (Mount Moosilauke, New Hampshire) were frequently submerged in clouds and exposed to the dissolved ions that are carried by the clouds and the annual nitrogen deposition was 16.3 kg NH_4^+ and 102 kg NO_3^- ha^{-1} (Lovett et al., 1982).

Recent studies support the hypothesis that leaf-absorbed nitrogen causes mineral element imbalances in coniferous trees. Hambruckers and Remacle (1991) detected higher NH_4^+, phosphorus, and potassium concentrations in chlorotic and magnesium-deficient needles of Norway spruce than in normal foliage. Douglas-fir foliage in western Washington retained about 90 % of the NH_4^+ from the precipitation water where the annual input was 4.5 kg N ha^{-1} (Van Miegroet and Cole, 1985). The absorption of the air-borne nitrogen seems to be dependent on the nitrogen concentration in the precipitation. In the Netherlands where the yearly deposition was as high as 40 to 60 kg N ha^{-1}, NH_4^+ concentrations collected under trees were higher than those in the precipitation, as NH_4^+ appeared to be leached from the crowns (Roelofs et al., 1985; Van Breemen et al., 1984).

Potassium in balsam fir from Mount Greylock ranged from 1.9 to 2.7 mg g^{-1} in the dry needles. Senser and Höpker (1989) identified potassium chlorosis of needles with these concentrations in Norway spruce. In one-year-old spruce needles of healthy trees, the potassium concentrations were described as being double those of calcium concentrations (Huntington et al., 1990; Kandler et al., 1987). The potassium concentrations in the balsam fir needles from Mount Greylock were lower than the calcium concentrations. A characteristic browning of the needle tips as a symptom of potassium deficiency described in Baule and Fricker (1967) was not apparent in the balsam firs. Magnesium between 0.4 and 0.6 mg g^{-1} in the one- and three-year-old needles of the balsam firs at Mt. Greylock (Table 3.3) was at the threshold of deficiency measured in standards obtained from spruce (Wittich, 1959; Zöttl, 1990). A large variability in mineral element concentrations occurred between neighboring trees. This variability makes it very difficult to draw conclusions about the health status and the impact of plant nutrients in mature forest trees (Krivian and Schaldach, 1986).

Magnesium deficiency combined with potassium deficiency in the balsam firs may be the causal factor for the nitrogen and chlorophyll loss in these chlorotic trees. Degradation of chlorophyll is generally due to protein loss during senescense (Allinger et al., 1969). The deficiency of mineral elements, e.g. magnesium, that regulate enzyme activity and anabolic processes in protein metabolism is an important cause for the deterioration of chlorophyll (Baszyski et al., 1980).

In coniferous trees, however, chlorophyll deterioration in older needles occurs naturally during spring when the new flush starts to grow, but older needles will regain green color when magnesium is delivered from the stem directly from the soil (Köstner et al., 1989; Lange et al., 1989). Köstner et al. (1989) reported that chlorophyll concentrations in spruce needles were dependent on magnesium concentrations, but in contrast needles with low chlorophyll content had no decreased photosynthetic rates than greener needles. This result suggested that chlorotic trees must not necessarly have decreased carbon assimilation as reported in a study of Mengel et al. (1988). Other workers suggested that injuries to the canopy due to acid rain affect photosynthate and mineral element allocation in forest trees. Applications of acidic mist (pH 1.7) have reduced photosynthesis in Norway spruce seedlings (Leisen and Marschner, 1990). Acidic mist induced restriction of phostosynthesis, reduced rates of carbohydrates transported into the roots and consequently restricted root growth and mineral nutrient uptake (Mengel, 1988).

The apparent magnesium and potassium deficiency in the balsam firs may have been due to low availability in the soil. In this case, the chlorosis could have been induced by magnesium translocation from the older to the younger needles as described previously. The soil analysis of the sites 1000 ft below the location of the balsam fir on the summit of Mt Greylock (Table 3.4. and 3.5.) showed a lesser abundancy of magnesium and potassium than calcium, so magnesium and potassium may be limiting factors in these soils. It can be suggested that the shallow soil at the mountain top provided even less mineral nutrients than the Spodosols downslope.

Low magnesium and potassium concentrations probably induced chlorophyll loss in three-year-old needles of balsam fir that was probably due to lessened protein synthesis. Magnesium and potassium deficiency was most likely induced by low concentrations in the soil. A contribution of nitrogenous compounds from cloudwater to foliar nitrogen is a possible explanation for relatively high nitrogen concentrations in the needles.

3. <u>Soil Profiles and Soil Chemistry</u>

The determination of the physical and chemical soil characteristics at the site in the Mount Greylock State Reservation provided a better understanding of the soil in which high elevation forest trees grow. The results obtained permit discussion of acidification, cation leaching, and mineral element deficiencies in forest soil and trees.

The properties of the described soil profiles (Table 3.4.) corresponded with the findings with other forested soils in New England. Dethier et al. (1988) reported similar values for acidity and calcium and potassium concentrations in a profile of a Haplorthod in Mount Greylock, State Reservation. Dethier et al. (1988) determined large amounts of soluble SO_4^{2-}, Al^{3+}, and dissolved organic carbon in the A and B horizons. They concluded that organic matter controlled the soil pH and the cation exchange capacity (CEC), which was 100 cmol kg^{-1} in the O horizon and 25 cmol kg^{-1} in the A horizon. The pH of the O-horizon in all soil samples (Table 3.6.) was one unit lower than in the A horizon where less organic material was present. In soils from New England studied by Federer and Hornbeck (1985), calcium occupied the largest portion of exchangable ions as was detected also in the soils from the site at Mount Greylock. The buffer capacity was about 100 meq kg^{-1} pH^{-1} in the organic matter, uniformally in New England forest floors (Federer and Hornbeck, 1985).

It is debated whether the acidity of the topsoil is due to the organic acids of the tree litter or if acidic precipitation acidifies soils. Krug and Frink (1983) ascribe the acidification of the soils and aquifers in New England to changes in land use. By the turn of the last century, farming in New England was abandoned, and reforestation took place. The altered vegetation had an important influence on soil acidity. Coniferous trees have a high capacity to release organic acids from their litter (Riha et al., 1986). The genesis of the spodosols that occurred at the studied forest sites can be explained as a function of parent material, vegetation, and climate (Buol et al., 1980).

Federer and Hornbeck (1985) determined that it would take approximately 40 years to decrease the pH in an entire profile by one unit with today's acidity in the precipitation and 1000 mm rainfall per year. In the eastern United States, soil acidification therefore may be due to soil borne factors. However, the atmospheric proton input in western Europe exceeds the internal proton source by far so that evidence was found that acid rain indeed acidified soils (van Breemen et al., 1984; Kuylenstierna and Chadwick, 1991). Molitor and Raynal (1982) reported leaching of ions in soils of a hardwood and a coniferous stand in the Adirondack Mountains of New York. The concentrations of sulphate, calcium, magnesium, and sodium in the water leaving the sola were higher than in the rain water.

The calcium concentrations in the organic soil and the A horizon (Table 3.5.) decreased slightly between April and November. The loss of calcium in the topsoil is probably due to mineralization of the decaying organic material. Mineral nutrients from the needle litter appear to remain in the O horizon. The soil analytical data do not provide sufficient information to draw the conclusion that calcium was washed to deeper horizons. The generally low exchangeable mineral element concentrations in the subsoil are due to the parent material, the degree of soil development of New England soils, and the low CEC that is reduced in low pH ranges (Veneman and Bodine, 1982; Federer and Hornbeck, 1985; Dethier et al., 1988).

Red spruce that grow in shallow soils which are low in mineral nutrients must use the available elements very efficiently to remain in healthy condition. The relatively nutrientrich O horizon appears to be an important pool for mineral elements in the nutrient cycle of forests.

CHAPTER IV

EXPERIMENTS WITH INDICATOR PLANTS TO ASSESS MINERAL NUTRITION IN ACID SOIL

A. Introduction

Nutrition of plants in the forest depends on internal nutrient cycling within the forest system, the release of mineral nutrients from the soil, and import of elements in precipitation. Organic material represents the major source of potassium, calcium, and magnesium, because mineral soil is low in nutrients and contributes little to the nutrition of forests (see Chapter III). Forest soils are highly acidic. In acid soils, calcium and magnesium become limiting to plants due to their low abundance in the soil and due to ionic competition at the root-soil interface (Arnon and Johnson, 1942). In low pH ranges, aluminum and manganese can be toxic to roots and inhibit mineral nutrient uptake in acid soils (Foy et al., 1978). Nutritional disorders in plants grown in acid soils have been studied thoroughly and, studies have employed the use of indicator plants to assess mineral element deficiencies, e.g., calcium deficiency (Jackson, 1967). Radishes appeared to be a good indicator plant because they grow fast and have a high calcium requirement (Barker et al., 1988).

Nitrogen nutrition may add another stress to plants. Ammonium is the predominant nitrogen form in acid soil because bacteria that oxidize NH_4^+ released from the organic matter are not funtional at low pH (Alexander, 1965).

Plants do not tolerate ammonium nutrition when pH in the medium is below 6.0 and when calcium supply is limited (Street and Sheat, 1958).

Two experiments were designed to study growth of radishes as indicator plants to assess the fertility of forest soils with respect to calcium and magnesium. Radishes have a high calcium requirement and are sensitive to form and amount of nitrogen (Barker et al., 1983; Barker et al., 1988). The first experiment evaluated the capacity of the organic horizon and the mineral horizon soil to provide calcium and magnesium. Liming treatments were used to study the effects of soil acidity on plant growth and mineral composition. In the second experiment, indicator plants were subjected to ammonium nutrition and amendments of calcium and magnesium to assess the capacity of NH_4^+ ions to affect calcium and magnesium accumulation in radishes grown in the soil from the organic horizon.

B. Materials and Methods

1. Nutrition of Indicator Plants in Organic and Mineral Horizons

Radishes (Raphanus sativus L.) cultivar Cherry Belle were seeded in soil from the O and A horizon of a Typic Haplorthod obtained from a hardwood-red spruce stand in Mount Greylock, State Reservation (see Chapter III). The soils were collected in bulk in October 1990 and stored in a dark, cold room until the experiment was started in December. Coarse organic material and stones were removed by hand before the soils were placed in 6-inch diameter x 4.5-inch deep pots (1.8 L). Radishes were sown 1 cm deep and were thinned gradually to five plants per pot.

After one week, the nutrient solution treatments were started (Table 4.1). Nutrient solutions were based on Hoagland's No. 1 solution (Hoagland and Arnon, 1950) with 10 mM NO_3. Treatment 1 was calcium- and magnesium-deficient. In treatments 2 and 3, the pots received a $CaCO_3$ amendment of 2.0 and 4.0 grams per pot, respectively. Treatment 4 and 5 received a 5.0 mM calcium chloride and 2.0 mM magnesium sulfate amendment, respectively. Nutrient solutions were applied daily in 100-ml portions for 28 days. After the end of the experiment the acidity of the soil in the pots was measured with a potentiometrically using a 1:1 and 1:5 soil-water suspension for A horizon and O horizon soil, respectively.

2. Effects of NH_4^+ on Growth and Mineral Element Composition of Indicator Plants

This experiment assessed the role of magnesium and calcium amendments in combination with ammonium nutrition of indicator plants. Radishes were grown in soil from the O horizon of a hardwood-red spruce stand (pH 3.6) and with a 2 g per pot $CaCO_3$ treatment. Ammonium was applied at 7.5, 10.0, 12.5, 15.0, or 17.5 mmol L^{-1} from a nutrient solution. Each plot was subdivided in three subplots with calcium in combination with magnesium (CA+MG), calcium alone (CA), or

Table 4.1. Composition of the nutrient solutions for nutrition of indicator plants in organic and mineral horizons.

	Treatment			
	1	2 and 3	4	5
Salts	(0)	$(+CaCO_3)^x$	(+Ca)	(+Mg)
	Concentration of the Salt (mM)			
$Ca(NO_3)_2$	-	-	5	-
KNO_3	5	5	-	5
KH_2PO_4	1	1	1	1
$NaNO_3$	5	5	-	5
KCl	-	-	5	-
$MgSO_4$	-	-	-	2

[x] Treatment 2, 2.0 g $CaCO_3$; treatment 3, 4.0 g $CaCO_3$ per pot.

magnesium alone (MG) applied in the nutrient solution. The concentrations were 5 mM calcium as $CaCl_2$ and 2 mM magnesium as $MgSO_4$. The other elements and micronutrients were applied as suggested for Hoaglands No. 1 nutrient solution (Hoagland and Arnon, 1950). The treatments were applied over a period of 28 days, and each pot received 1.2 liters of nutrient solution in total.

3. Statistical Design and Analysis

The first experiment had a split plot design with 4 replicates. The liming and the calcium and magnesium salt treatments were the main plots with the O and A horizon soil growth media as subplots. Data were processed by analysis of variance (ANOVA). The means were compared by least significant difference (LSD).

The second experiment had a split plot design with the ammonium treatments being the main plots and the calcium and magnesium treatments the split plots having five replicates. The data were processed by ANOVA. The effects of the ammonium treatments were assessed by regression analyses. Within ammonium treatments the calcium and magnesium treatments were compared by LSD.

C. Results

1. Nutrition of Indicator Plants in Organic and Mineral Horizons

Radishes germinated within 3 days in the acidic forest soils but exhibited yellowing of the cotyledons and death of the roots soon thereafter. To replace the dead plants, transplants of 8-day-old radish plants were taken from pots that had a $CaCO_3$ amendment. Growth of the transplanted radishes continued poorly in all pots without $CaCO_3$ treatment so that very little plant material was harvested in treatment 1, 4, and 5. Radishes grown in O horizon soil produced dry weights of 0.8 to 2.4 g per pot. In the pot filled with mineral soil, the radishes weighed between 0.6 and 1.8 g. The leaf blades did not expand and had a yellowish color except for the very green but small leaves of plants treated with magnesium. The samples of the two forest sites were combined so that four replicates for each treatment and soil type (A and O horizon) were obtained.

In treatment 1, where no calcium or magnesium was added, the acidity of the organic horizon soil was pH 3.9 and of the mineral horizon was pH 4.2. The 4 g $CaCO_3$ amendment increased the pH in the soil up to 5.2 in the O and A horizon. The soil pH was 3.6 in the O horizon and 3.8 in the A horizon in treatment 4 and 5.

Radishes in all treatments accumulated between 5.0 and 6.0 % total nitrogen with no apparent difference due to treatment. The treatments and the soil type had impacts on the potassium, calcium, and magnesium concentrations in the radishes (Table 4.2). Potassium concentrations were not affected by soil or its interaction with treatment. The treatments with 4 g $CaCO_3$ and with calcium salt amendment significantly increased potassium in radishes. Potassium concentrations in the 2 g $CaCO_3$ treatment were lower than

Table 4.2. Elemental composition of radish shoots grown in acid forest soil.

		Elemental Concentrations ($mg\ g^{-1}$ dry wt.)[x]		
Treatment	Horizon	K	Ca	Mg
1. no Ca or Mg	O	49^{b}	16^{d}	2.8^{c}
	A	46^{b}	24^{c}	2.2^{cd}
2. 2 g $CaCO_3$	O	42^{bc}	28^{b}	1.2^{e}
	A	35^{c}	29^{b}	1.0^{e}
3. 4 g $CaCO_3$	O	58^{a}	35^{ab}	1.9^{cd}
	A	46^{b}	37^{a}	1.5^{de}
4. + Ca	O	55^{ab}	27^{bc}	2.6^{c}
	A	52^{ab}	31^{b}	1.6^{d}
5. + Mg	O	47^{b}	15^{d}	4.5^{b}
	A	47^{b}	19^{cd}	7.1^{a}
Source		Results from ANOVA Significance of the F-Value		
Treatment(T)		*	**	***
Soil(H)		-	*	-
T * H		-	-	***

[x] Means followed by different letters within the columns are significantly different by LSD ($P \leq 0.05$).

*, **, and *** significant at $P \leq 0.05$, 0.01, and 0.001, respectively. -, non significant.

those in treatment 1 where no calcium or magnesium was added. The calcium concentrations in radishes grown in mineral soil were somewhat higher than those grown in the organic soil. In the limed treatments and the calcium salt treatment, calcium concentrations were 10 to 20 mg g^{-1} higher than in plants receiving the calcium-deficient treatments 1 and 5. Radishes accumulated high amounts of magnesium when grown in the A horizon and treated with magnesium than when grown in the O horizon or with other treatments. In all other treatments which received no supplementary magnesium, radishes had often higher magnesium concentrations if grown in the O horizon soil. $CaCO_3$ restricted accumulation of magnesium in the tissue generally below the concentrations in radishes in treatment 1.

2. Effects of NH_4^+ on Growth and Mineral Element Composition of Indicator Plants

After 25 days, all ammonium treatments induced toxicity symptoms in the plants receiving 5 mM calcium (CA) or 5 mM calcium and 2 mM magnesium (CA+MG). Radish leaves in the CA treatment were chlorotic, cupped, and very brittle. Radish leaves in the 2 mM magnesium (MG) treatment did not show any toxicity or deficiency symtoms. Radishes receiving the MG treatment exhibited some cupping and marginal chlorosis after 30 days only with the 17.5 mM ammonium treatment.

The results of the mineral analysis, ANOVA, and regression are shown in Tables 4.3, 4.4, and 4.5, respectively. The radish dry weights were highest in the MG

Table 4.3. Elemental composition and dry weights of radishes treated with different regimes of calcium, magnesium, and ammonium.

Treatment	Ammonium in Nutrient Solution (mM)				
	7.5	10.0	12.5	15.0	17.5
	Nitrogen (mg g^{-1} dry wt.)[x]				
MG	46^b	53^b	53^b	53^b	56^b
CA	58^a	60^a	57^a	61^a	66^a
CA+MG	57^a	59^a	60^a	60^a	64^a
	Potassium (mg g^{-1} dry wt.)				
MG	61^b	60^b	54^b	57^b	52^b
CA	79^a	77^a	81^a	83^a	82^a
CA+MG	74^a	82^a	80^a	81^a	85^a
	Calcium (mg g^{-1} dry wt.)				
MG	22^b	20^b	19^b	18^b	19^b
CA	31^a	28^a	30^a	29^a	24^a
CA+MG	33^a	29^a	28^a	27^a	24^a
	Magnesium (mg g^{-1} dry wt.)				
MG	5.4^a	5.2^a	5.7^a	5.8^a	5.5^a
CA	2.8^b	2.5^b	2.9^b	2.6^b	2.7^b
CA+MG	6.0^a	4.8^a	5.3^a	5.1^a	5.0^a
	Radish dry wt. (g pot^{-1})				
MG	3.1^a	3.5^a	3.7^a	4.1^a	3.6^a
CA	2.2^c	2.2^b	2.6^b	2.2^b	2.3^b
CA+MG	2.5^b	2.2^b	2.3^b	2.1^c	2.2^c

[x] Means within columns followed by different letters are significantly different by LSD ($P \leq 0.05$).

Table 4.4. Results of the ANOVA for elemental composition and dry weights of radishes treated with different regimes of calcium, magnesium, and ammonium.

Source	N	K	Ca	Mg	dry wt.
	Variable				
	Significance of the F-value				
NH_4^+ (A)	***	-	***	-	-
Ca and Mg(T)	***	***	***	***	***
A x T	-	-	-	-	**

*, **, and *** significant at P ≤ 0.05, 0.01, and 0.001. respectively. -, nonsignificant.

Table 4.5. Results of the regression analysis for effects of ammonium treatments on elemental composition and dry weights in radishes treated with Ca and Mg.

	VARIABLE				
	N	K	Ca	Mg	Dry wt.
	Significance of the Regression[w]				
	MG Treatment[x]				
linear	***	*	**	-	**
quadratic	-	-	**	-	**
cubic	**	-	-	-	-
	CA Treatment[y]				
linear	**	-	**	-	-
quadratic	**	-	-	-	-
cubic	-	-	-	-	-
	CA+MG Treatment[z]				
linear	**	*	***	**	-
quadratic	-	-	-	-	-
cubic	-	-	-	**	-

[w] *, **, and *** significant at $P \leq 0.05$, 0.01, 0.001, respectively. -, non significant.
[x] MG, 2 mM magnesium.
[y] CA, 4 mM calcium.
[z] CA+MG, 5 mM calcium and 2 mM magnesium.

treatment (Table 4.3) and were positively affected by higher NH_4^+ supply (Table 4.5) with this treatment. Dry weights in the CA+MG treatment were lower than those in the CA treatment when NH_4^+ amendment exceeded 12.5 mM (Table 4.3). The regression analysis for the dry weights in the CA and CA+MG treatment did not show significant trends indicating that ammonium treatments did not affect dry weights in those treatments (Table 4.4).

The nitrogen concentrations in the radishes increased with all three treatments (MG, CA, CA+MG) as NH_4^+ supplied in the nutrient solution increased (Table 4.3). Nitrogen was increased by 6 to 10 mg g^{-1} dry weight as the NH_4^+ concentration in the treatments increased from 7.5 to 17.5 mM NH_4^+ (Table 4.3). Nitrogen also was affected by the calcium and magnesium treatments (Tables 4.3 and 4.4). Nitrogen was higher in radishes supplied with calcium (CA and CA+MG) than in those supplied only with Mg. The potassium concentrations were higher in radishes grown with the CA and CA+MG treatments than in those grown with the MG treatment. The NH_4^+ treatment did not affect potassium concentrations in radishes in the CA treatment whereas some linear response of potassium was observed with the MG (decline) and MG+CA (increase) treatments (Tables 4.3 and 4.5).

With the CA and the CA+MG treatments, consistently higher calcium concentrations were detected compared to those in the MG treatment (Table 4.3.). The trend towards lower calcium concentrations with increasing ammonium supply is strongly linear in all three treatments (Table 4.5.). The calcium suppression due to ammonium was strongest with plants receiving supplementary calcium. The calcium concentrations decreased by 9.3 and 8.1 mg g^{-1} dry weight in the CA and CA+MG as opposed to 3 mg Ca g^{-1} dry weight in the MG treated radishes.

Magnesium concentrations in plants grown in the magnesium-deficient treatment (CA) reached about half the level of those treated with magnesium (Table 4.3.). Leaves in the MG and the CA+MG treatment had the same magnesium concentrations. The magnesium concentrations were affected by the NH_4^+ treatments (Tables 4.3 and 4.4), but most of this effect was due to the decreased magnesium concentrations occurring with the CA+MG treatment (Table 4.5.).

D. Discussion

Radish seedlings did not grow in the acid soil without liming or without supplementary $MgSO_4$ and $CaCl_2$. Transplanted young radish plants grew in acid medium after they had germinated in a $CaCO_3$ buffered soil. The primary roots apparently required high amounts of calcium in order for the seedling to become established. Transplanted radishes in the treatments with supplementary calcium or magnesium (treatment 4 and 5) continued growing in acid soil (pH < 4). The liming treatment in the second experiment decreased the acidity in the pots from pH 3.7 to pH 4.6 and permitted germination and establishment of young radish plants in a still acid medium. Hence it appears that calcium deficiency in the root zone and not acidity restricted plant growth, although acidity at an intensity below pH 4.0 can be phytotoxic (Arnon et al., 1942; Arnon and Johnson, 1942).

Calcium requirements for root and shoot growth are well studied. Coutts and Philipson (1980) reported a higher potential for growth restrictions of shoots and roots of Sitka spruce from calcium deficiency than from magnesium deficiency. Jackson (1967) observed that roots of barley and wheat did not grow in calcium-deficient media due to restricted cell division and elongation, and in acid soils potato stems died before they emerged (Jackson, 1967). Poor root growth can lead to further complications in plant nutrition. Marschner et al. (1991) estimated that 80% of the calcium in shoots of spruce is absorbed in the region of elongation at the root tip, where the endodermis is not yet formed. Calcium which is transported only in the transpiration stream would become deficient in the shoot as water uptake is diminished due to a decreased number of root tips and root surface (Kirkby and Pilbeam, 1984).

Barker et al. (1988) conducted a sand culture experiment with nutrient solutions comparable to those used in experiment 1 and 2. With 5 mM calcium and 2 mM magnesium in the treatments, radishes accumulated 32 mg K, 41 mg Ca, and 8 mg Mg g^{-1} dry weight. The critical calcium concentration, determined in the same study, at which deficiency symptoms were apparent with nitrate nutrition was 20 mg g^{-1} dry weight. According to these values, calcium was deficient in all radishes of the no calcium treatment and the magnesium treatment in the first experiment (Table 4.2) and with the magnesium salt treatment in the second experiment (Table 4.3). The $CaCl_2$ and the $CaCO_3$ amendments provided sufficient calcium for radishes, raising their calcium concentrations higher than 20 mg g^{-1} dry weight.

Radishes grown on magnesium-free medium contained around 2 mg Mg g^{-1} and exhibited chlorosis as a symptom of deficiency. The large increase in magnesium concentrations due to Mg amendments in acidic media demonstrated that acidity did not inhibit magnesium uptake if magnesium was present in sufficient amounts. Fertilization with magnesium can therefore improve growth conditions in acidic soils. Kandler et al. (1987) applied magnesium to chlorotic and magnesium-deficient Norway spruce and reversed the symptoms and allowed needles to become green again.

The potassium concentrations in the radishes from either experiment were above 55 mg g^{-1} dry weight. A depression of potassium accumulation due to ammonium nutrition as reported for bean, corn, cucumber, and pea (Maynard and Barker, 1969) did not occur in the radishes in the present study except in the MG treatment. Potassium does not appear to be limiting in the present study.

The second experiment investigated the response of the indicator plants to ammonium nutrition in acid soil (pH 4.6). An additional aspect of interest was the impact of liming and calcium salt amendment alone or in combination with a magnesium treatment on plant growth. This aspect was studied to demonstrate that deposition of air-borne NH_4^+ into forests has the potential to enhance growth and consequently lead to nutrient deficiencies. Due to limited calcium supply and buffer capacity of soils, toxic effects of NH_4^+ are possible even in low concentrations for field crops (Arnon et al., 1942; Street and Sheat, 1958) and forest trees (Evers, 1964; Ingestad and Kähr, 1985).

The plants in the CA and CA+MG treatment did not tolerate any of the ammonium treatments. The rolling of the leaf margins that developed in radishes 8 days after the ammonium treatments were started, resembled calcium deficiency symptoms (English and Maynard, 1978). Street and Sheat (1958) stated that plants utilize ammonium nitrogen without toxic effects only if the pH of the root zone is close to neutrality. Barker et al. (1966) showed that the transport of NH_4^+ from roots to shoots is restricted in neutral media. Ammonium becomes toxic when it enters the green plant tissue where it uncouples photosynthetic phosphorylation (Krogmann et al., 1959). Schenk and Wehrmann (1979) reported a restriction of root elongation and root hair formation due to ammonium nutrition and low calcium levels in cucumbers grown in nutrient solution.

Radishes receiving NH_4^+ nutrition exhibited deficiency symptoms in the present study although they contained between 25 and 35 mg Ca g^{-1} dry weight. These calcium concentrations are higher than the critical concentrations obtained from experiments with nitrate nutrition (Barker et al., 1988) but apparently were insufficient with NH_4^+ nutrition in the present study. Plants receiving calcium and magnesium salt treatment (CA+MG) became chlorotic due to the NH_4^+ treatment although they contained sufficient magne-sium and as much as radishes grown with magnesium alone (MG).

Oddly, radishes grown with $CaCO_3$ and magnesium salt amendment (MG) had calcium concentrations below 20 mg g^{-1} dry tissue and showed no toxic effects of ammonium even if the concentration in the nutrient solution was 17.5 mM NH_4^+. The calcium source for the plants with the magnesium treatment was the $CaCO_3$ added to establish the young radish plants. In case of a complete dissolution, the 2 g $CaCO_3$ would have released 800 mg of calcium. The calcium salt treatment in the other treatments (CA and CA+MG) supplied an additional 540 mg calcium per pot. These high amounts of calcium or the wide ratio between calcium and magnesium nutrition were somehow not beneficial to the radishes and enhanced the appearance of chlorosis and deficiency symptoms. The accumulation of chloride ions with $CaCl_2$ amendments of the CA and CA+MG treatments does not seem to be a source of salt damage, for leaves showed no symptoms of Cl^- toxicity (Eaton, 1966).

An impact of increased calcium supply on the accelerated appearance of ammonium toxicity symptoms has been reported. Barker et al. (1988) observed the best root growth in radishes grown on a $CaCO_3$ buffered NH_4^+ nitrogen nutrition when the calcium supplied in the nutrient solution was between 1 and 25 mg L^{-1}. Higher concentrations of calcium restricted growth of roots and shoots linearly up to a supply of 200 ppm Ca. Calcium concentrations in the shoots were increased when the calcium supply went up. Possibly elevated calcium supplies facilitated the entry of ammonium and enhanced the toxicity of ammonium.

Radishes in the magnesium treatment (MG) accumulated 1.5 to 2 times more dry weight than the plants in either of the other treatments. Radishes with higher dry weights absorbed more total nutrients than plants with lower dry weights. Hence the total accumulation of calcium in plants receiving liming, $MgSO_4$ and no $CaCl_2$ (MG treatment) was in some cases higher than in those supplied with $CaCl_2$ (CA and CA+MG). Whereas increasing NH_4^+ had a negative to no effect on the dry weights in the CA and CA+MG treatment, the plants in the MG treatment utilized the increased NH_4^+ amendments, yielding nitrogen concentrations and increased dry weights. A balanced nutrition with calcium and magnesium appeared to enhance the capacity of radishes to utilize ammonium as a nitrogen source.

The results obtained in the present chapter support the findings in chapter III, where it was stated that calcium and magnesium deficiencies in forest trees are caused by low mineral element concentrations and high acidity in the soil. It was shown experimentally that calcium and magnsium deficiencies in plants may be agravated if NH_4^+ is the nitrogen source. Ammonium has the potential to induce calcium and magnesium deficiencies even if calcium and magnesium are present in high concentrations in the growth medium and the plant tissue. In forest floors where calcium and magnesium reserves are limited, growth inhibition and chlorosis can be enhanced due to NH_4^+.

CHAPTER V

THE EFFECTS OF AMMONIUM ON MINERAL NUTRITION OF RED SPRUCE AND INDICATOR PLANTS

A. Introduction

Soil factors and chemical changes in the root zone are believed to contribute to nutritional disorders in declining forest trees (Schulze, 1989; Ulrich, 1990). Ammonium borne in the air and released from decaying organic matter may cause additional acidification beyond that which naturally occurs in forest soils and may compete for assimilation by the roots with other cations (Tjepkema, 1979; Nihlgard, 1985). The results of the mineral element composition of the foliage in forest trees (see Chapter III) showed that declining trees suffered nutrient deficiencies, which were related to needle loss with red spruce and chlorosis in balsam fir.

The effects of soil acidity and ammonium nutrition were studied previously with indicator plants (see Chapter IV). The experiments determined that acid forest soils did not provide sufficient calcium and magnesium for the growth of the indicator plants. Ammonium nutrition appeared toxic to plants and led to pronounced calcium deficiency in radishes even though calcium had been applied. The results obtained with indicator plants need to be related to red spruce to evaluate the potential contribution of NH_4^+ nutrition to mineral nutrient deficiencies in forest trees.

Ammonium supplied to indicator plants as a sole source of nitrogen in a unbuffered medium induced toxicity quickly (see Chapter IV). In the present study, therefore, ammonium was supplied in combination with nitrate. The greenhouse experiments in the present chapter assessed the effects of nitrogen form on growth and mineral nutrient composition of indicator plants and red spruce grown in acid O and A horizon soils from the forest.

B. Materials and Methods

1. Experiment with Indicator Plants

Radishes were seeded into 6-inch diameter pots filled with O or A horizon soils collected in bulk from a hardwood-spruce stand and a pure red spruce stand at Mount Greylock State Reservation. Because radishes did not grow without liming, 2 g $CaCO_3$ were added to each pot. After germination, plants were thinned to five plants per pot. Nutrient solution treatments started 7 days after emergence. Radishes were treated on a daily basis with 100 ml nutrient solution (Table 5.1.) and watered with deionized water. The nutrient solutions contained 15 mM nitrogen and consisted of 0, 3.75, 7.5, 11.25, and 15 mM NH_4^+ with the remainder supplied by NO_3^-. After 30 days of treatments, radish leaves were harvested, dried, weighed, and ground for mineral analysis. Nitrogen was determined by Kjeldahl analysis. Potassium, calcium, magnesium, and manganese were determined in dry ashed samples by atomic absorption spectrophotometry.

Table 5.1. Composition of nutrient solutions used in experiments with red spruce seedlings and radishes.

	Treatment Number, Nitrate:Ammonium Ratio				
	1	2	3	4	5
Salt	100:0	75:25	50:50	25:75	0:100
	concentration of the salt (mM)				
$(NH_4)_2SO_4$	0	1.88	3.75	5.63	7.5
$Ca(NO_3)_2$	5	5	3.75	0	0
KNO_3	5	1.25	0	3.75	0
K_2SO_4	0	1.88	2.5	0.62	2.5
$CaCl_2$	0	0	1.25	5	5
$MgSO_4$	2	2	2	2	2
KH_2PO_4	1	1	1	1	1

2. Experiment with Red Spruce Seedlings

One-year-old red spruce seedlings were obtained from the Spruce-Fir Research Cooperative, USDA Forest Service Durham, New Hampshire. To produce these seedlings, red spruce seeds had been collected in Carroll County near Chatham and Grafton County in the Waterville Valley in 1984. The seedlings were planted into 6-inch diameter pots filled with O and A horizon soils collected in bulk from a hardwood-spruce stand and a pure spruce stand at Mount Greylock, State Reservation in October of 1990. Seedlings were kept outside the greenhouse from October to January to encourage vernalization. The red spruce seedlings were moved into the greenhouse on January 20, 1991. The plants were watered with deionized water until the buds broke. The nutrient solution treatments (Table 5.1.) were started on February 1 and ended on May 24 (115 days).

Every third day, 100 ml of nutrient solutions were applied during the growth period of 115 days. Nitrogen supply per plant totaled 800 mg per pot. Trees were watered daily with deionized water and were flushed every two weeks with tapwater to limit the development of salt crusts on the soil surface in the pots. After the end of the experiment, the new growth was harvested and stored frozen. The dry weights of the remaining shoots were determined. The pH in the soil of every pot was measured potentiometrically in the O and A horizon soil in a 1:5 and a 1:1 soil-water suspension, respectively. Upon thawing chlorophyll was extracted from the new growth (Holden, 1965). The samples then were dried for mineral analysis.

3. Statistical Design and Analysis

The experiments were set up in a randomized complete block split plot design. The treatments of nutrient solution were the main plots. Forest soil origin (forest stands) and soil horizon (O and A) were the split plots. Each treatment in the split plots was replicated five times. The data were processed by analysis of variance (ANOVA). The effects of the nutrient solution treatments were assessed also by regression analysis. The means were compared by least significant difference (LSD).

C. Results

1. Experiment with Radishes Grown in Organic and Mineral Horizons and Treated with Different Ratios of NO_3^- and NH_4^+

After 28 days of treatment with the nutrient solutions that contained more than 11.25 mM NH_4^+, radish leaves became increasingly chlorotic with upward rolling of the leaf margins. Radishes grown with nitrogen treatments that contained more NO_3^- than NH_4^+ appeared dark green. Plants grown in O horizon soil developed bigger leaves than those grown in the A horizon soil.

a. Effects of Soil Horizon and Origin on Indicator Plants

Dry weights of radishes grown in O horizon soil were 1 to 2 g higher than those grown in A horizon soil (Tables 5.2 and 5.3). Nitrogen and potassium concentrations were higher in plants grown in the A horizon soils than in those grown

Table 5.2. Dry weights and elemental concentrations of radishes treated with different ratios of NO_3^- and NH_4^+ and grown in O and A horizons from two forest stands.

Soil		Nitrogen Treatment[x]					Nitrogen Treatment				
Horizon	Stand[y]	1	2	3	4	5	1	2	3	4	5
		Dry Weight ($g\ pot^{-1}$)[z]					Nitrogen ($mg\ g^{-1}$ dry wt.)				
O	HS	4.9^b	5.5^a	5.5^a	5.2^a	4.7^a	41^b	39^c	41^b	39^b	40^{ab}
	S	5.7^a	5.2^a	5.2^a	5.0^a	4.3^a	41^b	42^b	41^b	37^b	39^b
A	HS	3.0^d	3.4^b	3.5^b	3.1^b	2.6^c	45^a	43^{ab}	46^a	42^{ab}	41^a
	S	3.5^c	3.7^b	3.5^b	2.9^b	3.4^b	46^a	45^a	47^a	43^a	41^a
		Potassium ($mg\ g^{-1}$ dry wt.)					Calcium ($mg\ g^{-1}$ dry wt.)				
O	HS	25^c	24^b	25^c	25^b	28^b	38^c	37^{ab}	39^a	42^a	40^a
	S	34^b	26^b	26^c	27^b	30^b	30^d	32^b	34^b	38^b	35^b
A	HS	40^a	34^a	37^b	36^a	42^a	47^a	41^a	40^a	41^{ab}	38^{ab}
	S	42^a	35^a	42^a	38^a	42^a	43^b	41^a	38^a	43^a	38^{ab}
		Magnesium ($mg\ g^{-1}$ dry wt.)					Manganese ($mg\ g^{-1}$ dry wt.)				
O	HS	6.9^a	5.6^b	6.6^a	6.1^a	7.1^a	1.4^a	1.1^a	1.4^a	1.4^a	1.5^a
	S	5.7^c	6.6^a	6.8^a	6.4^a	6.0^b	0.4^b	0.5^b	0.5^b	0.6^b	0.5^b
A	HS	6.4^b	5.9^b	5.5^b	5.3^b	6.2^b	0.2^c	0.1^c	0.2^c	0.2^c	0.2^c
	S	5.6^c	6.0^b	4.7^c	5.1^b	6.0^b	0.1^c	0.1^c	0.1^c	0.1^c	0.1^c

[x] Treatment 1, 2, 3, 4, and 5: $NO_3^-:NH_4^+$ ratio of 15:0, 11.25:3.75, 7.5:7.5, 3.75:11.25, 0:15 mM, respectively.
[y] HS: hardwood spruce forest, S: red spruce stand.
[z] In columns means of dry weights and elements followed by different letters are significantly different by LSD ($P \le 0.05$).

Table 5.3. Results of ANOVA of dry weights and elemental concentrations in radishes treated with different ratios of NO_3^- and NH_4^+ and grown in O and A horizons from two forest stands.

Source	Significance of the F-Value					
	Dwt	N	K	Ca	Mg	Mn
N-Trmt(T)	***	***	***	**	-	**
Horizon(H)	***	***	***	***	***	***
Stand(S)	-	-	-	*	-	***
T*H	-	-	*	**	*	*
T*S	-	-	-	-	-	*
H*S	-	-	-	*	-	***
T*H*S	*	-	-	-	*	*

*, **, and *** are significant for $P \leq 0.05$, 0.01, 0.001, respectively. -, nonsignificant.

in the O horizon soils (Tables 5.2 and 5.3). Differences in nitrogen between the O and A horizon soil were 4 mg N g^{-1} with 15 mM NO_3^- (treatment 1) and greater than in those with 15 mM NH_4^+ (treatment 5) that were 1.6 mg N g^{-1}.

Differences in soil horizon and soil origin gave significant differences for potassium accumulation (Tables 5.2 and 5.3). Potassium was higher in plants grown in A horizon soil. The soil origin had little or no impact on plant potassium concentrations (Table 5.3).

Calcium concentrations differed between soil horizon and soil origin, and also a significant interaction of soilhorizon and nitrogen treatment occurred (Tables 5.2 and 5.3). For example with treatment 1, plants grown in soil from the mineral horizon from the hardwood-spruce forest contained 4 mg Ca g^{-1} more than those grown in A horizon soil from the spruce stand. With treatment 1 and O horizon soil from the red spruce stand, calcium concentrations in leaves were 8 mg g^{-1} lower than in those grown in O horizon soil from the hardwood-spruce stand. In the organic soil from the hardwood-spruce stand, calcium concentrations were also higher with treatment 2, 3, 4, and 5 than in the organic soil from the spruce stand. Calcium concentrations were higher in leaves of plants grown in A horizon soil than in plants grown in O horizon soil.

Higher magnesium concentrations occurred in radishes grown in O horizon soil than in radishes grown in the A horizon soils. (Tables 5.2 and 5.3). Origin of soil had no effect on magnesium accumulation by radish. A significant interaction of nitrogen treatment and soil horizon indicated a small increase in magnesium as a higher proportion of ammonium was included for plants grown in O horizons (Tables 5.2 and 5.3).

Differences between soil horizons were observed for manganese concentrations. The plants grown in the O horizon had more manganese than those grown in A horizon (Table 5.2). The highly significant effect of soil origin was due largely to the much higher accumulation of manganese by plants grown in the O horizon soil from the hardwood-spruce stand (Tables 5.2 and 5.3).

b. Effects of the Nitrogen Treatments on Indicator Plants

The effects of the nitrogen treatments on radish dry weights and mineral element compositions were highly significant ($P \leq 0.01$ or $P \leq 0.001$) for dry weights, nitrogen, potassium, calcium, and manganese (Table 5.3.). The treatment effect was not significant for magnesium.

The decrease in dry weight between treatment 1 (15 mM NO_3^-) and treatment 5 (15 mM NH_4^+) was significant in all soils (Table 5.4). The decline of the dry weights for plants grown in O and A horizon soil from the hardwood-spruce stand was curvilinear (quadratic), whereas the dry weights decreased linearly with radishes grown in O horizon soil from the red spruce stand (Table 5.2 and 5.4).

The decreases in nitrogen concentrations in radishes grown in O horizon soil from the red spruce stand and in

Table 5.4. Regression analysis for elemental concentrations against nitrogen treatments in radishes grown in O and A horizon soils from two forest stands.

	Significance of Regression					
	Dry wt.	N	K	Ca	Mg	Mn
	O Horizon from Hardwood-Red Spruce Stand					
linear	-	-	-	*	-	*
quadratic	***	-	***	-	*	-
cubic	-	-	-	-	-	*
	O Horizon from Red Spruce Stand					
linear	***	***	-	***	-	***
quadratic	-	-	***	-	***	*
cubic	*	**	-	*	-	*
	A Horizon from Hardwood-Red Spruce Stand					
linear	-	**	-	**	-	-
quadratic	**	-	**	-	**	-
cubic	-	-	-	-	-	-
	A Horizon from Red Spruce Stand					
linear	-	**	-	*	-	-
quadratic	-	-	-	-	**	-
cubic	*	-	-	-	**	**

*, **, and *** significant at $P \leq 0.05$, 0.01, and 0.01, respectively. -, non significant.

radishes grown in mineral horizon soil from either stand was linear with increasing ammonium in the nitrogen treatment. Although these trends were significant, the absolute changes were small. Nitrogen concentrations of plants grown in O horizon soil were decreased by only 1 or 2 mg N g^{-1} dry weight as ammonium in the nutrient solution was increased to 15 mM (Table 5.2). Nitrogen in the A horizon soils from either stand decreased linearly from 45 or 46 mg g^{-1} to 41 mg g^{-1} dry weight (Table 5.2 and 5.4). In the O horizon soil from either stand and the A horizon soil from the hardwood-spruce stand, potassium concentrations decreased between treatment 1 and 2 and went up as the NH_4^+ concentration in the nutrient solution was higher than 7.5 mM NH_4^+ (Treatment 3) (Table 5.2). Even though the regression were significantly quadratic (Table 5.4) the actual changes were small (Table 5.3).

Calcium concentrations were not greatly affected by the nitrogen treatment. For radishes grown in O horizon soil from the spruce stand, calcium increased linearly as NH_4^+ in the nutrient solution increased (Table 5.2 and 5.4). On the other hand, calcium concentrations decreased significantly by 5 or 9 mg g^{-1} in radishes grown in A horizon soil as NH_4^+ was increased (Table 5.2).

Magnesium concentrations were affected variably. Regressions of magnesium against nitrogen treatment were significant but not protected (Tables 3.3 and 3.4). In organic soil from the red spruce forest, magnesium increased with increasing NH_4^+, whereas radishes grown in O or A horizon soil from the hardwood-spruce stand had decreased magnesium with increased NH_4^+ (Tables 5.2 and 5.4).

Nitrogen treatments had little effect on the manganese concentrations in radishes except for plants grown in O horizon from the spruce stand (Table 5.4). Although the regression was significant, the concentration differences between the treatments were so small as to be considered biologically negligible (Table 5.2 and 5.4).

2. <u>Experiment with Red Spruce Seedlings Grown in Organic and Mineral Horizons and Treated with Different Ratios of NO_3^- and NH_4^+</u>

From the terminal buds of the red spruce seedlings, 3 to 4 cm long extensions grew after the plants were brought from the cold to the greenhouse. The seedlings had similar heights of 38 to 45 cm. After 100 days of nutrient solution treatment, needles of the new growth in the treatments with higher than 11.25 mM NH_4^+ became increasingly chlorotic. Seedlings grown with nutrient solutions in which NO_3^- dominated were dark green.

a. Effects of Soil Horizon and Origin on Spruce Seedlings

Growth restrictions of seedlings in the A horizons were observed with lower dry weights of the stems and needles than with plants grown in the O horizon (Table 5.5). The soil had a highly significant effect on needle growth but no effect on stem weight (Table 5.7). Stem and needle weights were highest with treatments 1, 2, or 3 and generally

Table 5.5. Dry weights of red spruce seedlings, needles and pH in the soil medium after the end of the experiment.

Soil		Nitrogen Treatment[x]				
Horizon	Stand[y]	1	2	3	4	5
		Dry weight of Seedlings (g)[z]				
O	HS	4.3[b]	4.7[b]	5.3[a]	4.2[a]	3.7[a]
	S	5.6[a]	5.4[a]	5.7[a]	3.5[b]	3.2[b]
A	HS	3.5[c]	4.1[c]	4.0[b]	3.2[b]	3.7[a]
	S	4.4[b]	4.9[b]	3.9[b]	4.6[a]	2.9[b]
		Dry Weight of 1991 Needles (g)				
O	HS	4.1[b]	3.3[ab]	2.6[b]	2.6[a]	2.9[a]
	S	4.9[a]	3.5[a]	3.3[a]	1.8[b]	2.0[b]
A	HS	3.1[c]	3.3[ab]	1.4[c]	1.7[b]	1.6[c]
	S	3.0[c]	3.1[b]	1.6[c]	1.3[c]	1.1[c]
		pH in the Soil Medium				
O	HS	3.5[ab]	3.4[b]	3.5[b]	3.5[ab]	3.3[b]
	S	3.4[b]	3.3[b]	3.3[c]	3.5[ab]	3.1[b]
A	HS	3.5[ab]	3.3[b]	3.5[b]	3.4[ab]	3.4[ab]
	S	3.6[a]	3.6[a]	3.7[a]	3.6[a]	3.5[a]

[x] Treatment 1, 2, 3, 4, and 5: NO_3^-:NH_4^+-ratio of 15:0, 11.25:3.75, 7.5:7.5, 3.75:11.25, 0:15 mM, respectively.
[y] HS, hardwood spruce stand; S, red spruce stand.
[z] Means in columns followed by different letter are significantly different by 0.05, LSD.

Table 5.6. Elemental concentrations in red spruce treated with different ratios of NO_3^- and NH_4^+ and grown in O and A horizons from two forest stands.

		Nitrogen Treatment[x]					Nitrogen Treatment				
Horizon	Stand[y]	1	2	3	4	5	1	2	3	4	5
		Nitrogen (mg g^{-1} dry wt.)					Potassium (mg g^{-1} dry wt.)				
O	HS	23^{ab}	26^{a}	22^{a}	19^{b}	19^{a}	12^{a}	9^{a}	9^{a}	11^{a}	13^{ab}
	S	26^{a}	27^{a}	23^{a}	24^{a}	21^{a}	12^{a}	11^{a}	9^{a}	13^{a}	16^{a}
A	HS	21^{b}	19^{b}	20^{a}	19^{ab}	18^{a}	14^{a}	11^{a}	11^{a}	10^{a}	12^{b}
	S	21^{b}	23^{ab}	19^{a}	18^{b}	20^{a}	13^{a}	12^{a}	10^{a}	10^{a}	12^{b}
		Calcium (mg g^{-1} dry wt.)					Magnesium (mg g^{-1} dry wt.)				
O	HS	5.6^{b}	6.9^{a}	5.1^{a}	5.0^{a}	5.2^{a}	1.5^{a}	2.0^{a}	1.9^{a}	1.9^{a}	2.4^{b}
	S	6.1^{b}	6.4^{a}	5.6^{a}	5.2^{a}	4.0^{a}	1.9^{a}	1.9^{a}	1.8^{a}	2.1^{a}	2.6^{ab}
A	HS	6.6^{ab}	5.3^{a}	5.0^{a}	4.9^{a}	4.8^{a}	1.9^{a}	1.5^{b}	1.8^{a}	2.0^{a}	2.1^{b}
	S	7.9^{a}	6.7^{a}	4.4^{a}	4.4^{a}	4.7^{a}	1.9^{a}	2.0^{a}	2.1^{a}	2.0^{a}	2.9^{a}
		Manganese (mg g^{1} dry wt.)									
O	HS	2.2^{a}	2.8^{a}	2.6^{a}	2.4^{a}	2.5^{a}					
	S	1.4^{ab}	1.6^{b}	2.2^{a}	1.4^{b}	1.4^{b}					
A	HS	0.8^{b}	0.6^{c}	0.9^{b}	0.7^{bc}	0.7^{b}					
	S	0.7^{b}	0.7^{c}	0.8^{b}	0.5^{c}	0.7^{b}					

[x] Treatment 1, 2, 3, 4, and 5: $NO_3^-:NH_4^+$ ratio of 15:0, 11.25:3.75, 7.5:7.5, 3.75:11.25, 0:15 mM, respectively.
[y] HS, hardwood spruce stand; S, red spruce stand.
[z] In columns means of elements followed by different letter are significantly different by LSD ($P \leq 0.05$).

Table 5.7. Results of the AVOVA for red spruce seedlings treated with different ratios of NO_3^- and NH_4^+ and grown in O and A horizons from two forest stands.

	Significance of the F-Value[v]									
Source	N	K	Ca	Mg	Mn	Chla[x]	Chlb	Dwtn[y]	Dwts[z]	pH
N-Trmt(T)	**	***	***	***	*	***	***	**	*	***
Horizon(H)	*	-	-	-	***	-	-	***	-	**
Stand(S)	*	-	-	-	*	-	-	-	-	-
T*H	-	*	-	-	-	-	-	-	-	***
T*S	-	-	-	-	-	**	**	-	-	***
H*S	-	-	-	-	-	-	-	-	-	**
T*H*S	-	-	-	-	-	*	-	-	-	-

[v] *, **, and ***, significant at $P \leq 0.05$, 0.01, and 0.001, respectively. -, non significant.
[x] Chlorophyll determined only for teatment 3, 4,and 5.
[y] Total dry weights of 1991 grown needles.
[z] Dry weights of stems.

declined with treatment 4 and 5. No interaction of treatments had affected growth (Table 5.7).

The acidity in the soil medium at the end of the experiment was between pH 3.1 and 3.5 in the O horizon soils and between pH 3.3 and 3.7 in the A horizon soil from the spruce stand. These values, however, were relatively unchanged from the original pH 3.4 to pH 3.9 (Tables 5.5 and 3.4). The treatment x soil interaction and the location x soil interaction indicated that pH fell more in the O horizon than in the A horizon and more in soil from the spruce stand than in soil from the hardwood-spruce stand (Table 5.7).

Amongst the mineral element concentrations determined, only nitrogen and manganese in needles differed with soil horizon or soil origin (Tables 5.6 and 5.7). Seedlings grown in O horizon soil had higher nitrogen concentrations than those in the A horizon. Needles of plants grown insoil from the spruce stand had higher nitrogen concentrations than those from the hardwood-spruce soil. Potassium concentrations were in a range of 9 and 16 mg g^{-1} and were not different between soil horizons or between soil origins(Tables 5.6 and 5.7). Magnesium concentrations were not affected by the soils (Table 5.6 and 5.8).

Through all five nitrogen treatments, manganese concentrations were higher for seedlings grown in O horizon than in the A horizon (Table 5.6). Seedlings tended to accumulate more manganese if grown in the soil from the hardwood-spruce stand. Chlorophyll a concentrations were 3 to 4 times higher than chlorophyll b concentration (Table 5.8). Horizons or origin of soil did not effect chlorophyll concentrations (Table 5.7).

b. Effects of the Nitrogen Treatments on Spruce Seedlings

Significant effects of nitrogen treatment were observed for the dry weights, the soil pH, and all plant components (Table 5.7). The regression analysis assessed the effects of the nutrient solution treatments for each growth medium separately (Table 5.9).

Dry weights of the stems and needles decreased as NH_4^+ in the nutrient solution went up (Table 5.5). The treatment effect was more significant on needle dry weights than on stem dry weights for stem weights (Tables 5.7 and 5.9). In seedlings grown in organic horizons, 1.3 to 1.5 g less needle weight was accumulated in the 15 mM NH_4^+ treatment than in the 15 mM NO_3^- treatment, whereas this difference was 0.5 to 1.9 g for the A horizon soil (Table 5.5). The decline in dry weights of the needles was linear with all growth media except for the O horizon soil of the hardwood-spruce stand (Table 5.9). The soil acidity had a slight tendency towards decreased pH with higher ammonium ratios but did not fit a common regression type (Tables 5.5 and 5.9).

In seedlings grown in O horizon soils, nitrogen concentrations increased slightly if the nutrient solution contained 25 % NH_4^+ (treatment 2) and decreased linearly

Table 5.8. Chlorophyll concentrations in needles of red spruce seedlings treated with three different ratios of NO_3^- and NH_4^+ nutrition and grown in O and A horizon from two forest stands.

Soil		Nitrogen Treatment[x]		
Horizon	Stand[y]	3	4	5
		Chlorophyll a (mg g^{-1} fresh wt.)[z]		
O	HS	2.3[a]	1.4[a]	1.5[a]
	S	2.1[a]	1.4[a]	1.5[a]
A	HS	2.0[a]	1.6[a]	1.2[a]
	S	1.8[a]	1.3[a]	1.4[a]
		Chlorophyll b (mg g^{-1} fresh wt.)		
O	HS	0.7[a]	0.5[a]	0.4[ab]
	S	0.6[a]	0.4[a]	0.4[ab]
A	HS	0.6[a]	0.5[a]	0.3[b]
	S	0.6[a]	0.4[a]	0.5[a]

[x] Treatment 3, 4, and 5: $NO_3^-:NH_4^+$-ratio of 7.5:7.5, 3.75:11.25, and 0:15 mM, respectively.
[y] HS: hardwood spruce forest, S: red spruce stand.
[z] Means for chlorophyll a or b followed by different letter are significantly different by LSD ($P \leq 0.05$).

Table 5.9. Results of regression analysis against nitrogen treatments for red spruce seedlings grown in O and A horizon from two forest stands.

		Significance of the Regression[v]							
Stand[x]		N	K	Ca	Mg	Mn	Needles[y]	Stems[z]	pH
					O Horizon Soil				
HS	linear	**	-	-	**	-	-	-	**
	quadratic	-	**	-	-	-	-	-	**
	cubic	**	-	-	-	-	-	-	**
S	linear	*	*	**	**	-	**	-	-
	quadratic	-	**	-	-	-	-	-	-
	cubic	-	-	-	-	-	-	-	*
					A Horizon Soil				
HS	linear	*	-	**	-	-	**	-	-
	quadratic	-	*	-	-	-	-	-	-
	cubic	-	-	-	-	-	-	-	*
S	linear	-	-	**	*	-	**	-	-
	quadratic	-	-	-	-	-	-	-	**
	cubic	-	-	-	-	-	-	-	-

[v] *, **, and ***, significant at $P \le 0.05$, 0.01, 0.001, respectively.
-, nonsignificant.
[x] HS, hardwood-red spruce; S, red spruce stand.
[y] Dry weight basis.
[z] Dry weight basis.

as the NH_4^+ increased (Tables 5.6 and 5.9). Nitrogen concentrations between treatment 1 and 5 decreased by 4 and 5 mg g^{-1} with the O horizon soils, but the decline was not significant for seedlings grown in the A horizon soil.

Potassium concentrations were higher in the treatments with 15 mM NO_3^- and 15 mM NH_4^+ than in treatments in which a combination of the two nitrogen sources was supplied (Table 5.6). This response fitted a quadratic regression and was significant for seedlings grown in O horizon soils and the A horizon soil from the hardwood-spruce stand (Table 5.9). Between the lowest and the highest NH_4^+ treatments, calcium concentrations decreased by 0.5 to 3.2 mg g^{-1} (Table 5.6). The regression was linear with all soil types and origins except for the seedlings grown in O horizon soil from the hardwood-spruce stand (Table 5.9).

The magnesium concentrations in the seedlings increased linearly as the NH_4^+ ratio in the nutrient solution rose (Tables 5.6 and 5.9). The differences between the concentrations with the 15 mM NO_3^- and the 15 mM NH_4^+ treatment for seedlings grown in the O horizon were 0.9 and 0.7 mg g^{-1}, and in the A horizon from the spruce stand the increase was 1 mg g^{-1}. The regression was not significant for seedlings grown in mineral horizon from the hardwood-spruce stand (Tables 5.6 and 5.9). Manganese concentrations were little affected by the nutrient solution treatments (Table 5.6 and 5.9).

Chlorophyll a concentrations decreased from 2.3 mg g^{-1} fresh weight to 1.4 mg g^{-1} fresh weight in the seedlings grown in O horizon soil from the hardwood-spruce stand. The decrease was between 0.4 and 0.6 mg g^{-1} fresh weight for the other growth media (Table 5.8). Concentrations of chlorophyll b also decreased between treatment 3 and 4. Needles of seedlings grown in A horizon soil from the hardwood-spruce stand had lower chlorophyll a and b concentrations with treatment 5 than with the other treatments. The regressions for declines in chlorophyll a and b in the organic soil grown seedlings was highly significant ($P \leq 0.01$). For seedlings grown in A horizon soil, the linear regression was significant ($P \leq 0.05$).

3. Summarized Results

a. Effects of soil horizon

Dry weights of radishes grown in A horizon were lower than those of plants grown in O horizon. Nitrogen, potassium, or calcium concentrations were somewhat higher in radishes grown in A horizon soil than in O horizon soil. Magnesium and manganese concen-trations were higher in radishes grown in O horizon soil. Spruce also had lower dry weights when growing in A horizon than those growing in O horizon. Soil horizon had no effect on nitrogen, potassium, calcium, and magnesium concentra-tions in spruce seedlings.

b. Effects of soil origin

Manganese concentrations were higher in plants grown in soil from the hardwood-spruce stand. Soil origin had little effect on nitrogen, potassium, calcium, and magnesium concentrations in foliage of radishes and spruce seedlings.

c. Effects of nitrogen treatments

Radishes grew better in a 1:1 mixture of NO_3^- and NH_4^+. Nitrogen treatment had no major effect s on elemental concentrations in radish leaves. Spruce seedlings had decreased needle growth with 3.75 mM NH_4^+ or more in the nutrient solution. Increasing NH_4^+ concentrations in the nutrient solution caused a decreasing trend for nitrogen and calcium concentrations in spruce seedlings. Magnesium concentrations in spruce seedlings were increased with higher concentrations of NH_4^+ in the nutrient solution.

D. Discussion

The objective of the present greenhouse study was to find evidence for the contribution of NH_4^+ nutrition in acid soil to mineral nutrient deficiencies. The focus of the study in particular was on the possible restriction of calcium and magnesium accumulation in red spruce seedlings and indicator plants brought about by increased NH_4^+ concentrations in the environment.

Plants treated with ammonium concentrations higher than 11.25 mM became chlorotic and exhibited restricted growth. Growth restriction and chlorosis in seedlings and indicator plants were clearly caused by the NH_4^+ treatment as shown by the significant decrease of dry weights and chlorophyll. Upon absorption of NH_4^+, protons are excreted into the rhizosphere by roots of conifer seedlings (Roelofs et al., 1985; Gijsman, 1990a). Rollwagen and Zasoski (1988) reported a decrease of pH from 5.7 to 4.7 in soil where Douglas-fir seedlings and mature trees were treated with NH_4^+ and observed the acidifying reaction to a soil depth of 20 cm in the forest. The liming requirement arising from NH_4^+ nutrition of forest trees can be high. For a pine forest in the Netherlands with an estimated annual needle production of 2000 kg ha^{-1}, Van Diest (1989) calculated an excretion of 3 keq H^+ which results in a need of 50 kg $CaCO_3$. Leisen et al. (1990) identified a small region of high proton efflux beginning at 0.5 cm behind the root tip when NH_4^+ was absorbed by fine roots of Norway spruce. The decrease in acidity of the bulk soil in the present study was relatively little because nitrogen accumulation in the plants was little because the acidification was restricted to a small soil portion in the immediate surroundings of the fine roots, and because the seedlings absorbed only a small portion of the nitrogen applied. From the seedlings, 1 to 5 g dry needles were harvested. Nitrogen was utilized by the seedlings with less than 10 % efficiency. Needles of seedlings contained 20 mg N g^{-1}, and the 0.8 g N applied could have produced 20 g dry weight.

Grasses and plants adapted to acid soils (calcifuges) have been reported to grow better with nitrogen supplied by a combination of both nitrogen forms or to be not capable of utilizing NO_3^- (Kirkby and Pilbeam, 1984; Burström, 1968). McElhannon and Mills (1978) studied nitrogen nutrition of lima beans in nutrient solution culture with five different ratios of NO_3^- and NH_4^+. They reported significantly decreased dry weights of roots and shoots as the NH_4^+ concentration in the nutrient solution exceeded 50 % of the total nitrogen supplied. Radishes prefer nitrogen in form of NO_3^- over NH_4^+ (Barker et al., 1988), whereas spruce are generally described to prefer NH_4^+ nutrition (Van Diest, 1989). Marschner et al. (1991) studied the preference of Norway spruce roots for nitrogen form. They observed that NO_3^- uptake was low but was not inhibited by NH_4^+ in the solution and detected a rapid depletion of NH_4^+ in the medium. The spruce seedlings in the present study exhibited a higher sensitivity to ammonium than radishes. Radishes were supplied with 2 g $CaCO_3$ to enable growth in the acid soil medium. Due to the buffering action of $CaCO_3$, radishes apparently utilized NH_4^+ until the NH_4^+/NO_3^- ratio exceeded 50% in the nutrient solution. The pH has to be close to neutrality for efficient nutrition of plants with NH_4^+ (Street and Sheat, 1958; Marschner, 1986), a requirement which was not fulfilled in the present experiment. After application of 2 g $CaCO_3$, soil acidity in the pots was still below pH 5.

According to the statistical results, calcium concentrations in red spruce seedlings and radishes were affected by the nutrient solution treatments. For seedlings, calcium concentrations decreased linearly whereas those of radishes were unchanged or had even increasing trends depending on the soil medium. Calcium concentrations in radishes were high compared to the results by Barker et al. (1988) who used soils with no lime or other Ca amendments and were not affected by the NH^+ treatment. Red spruce seedlings which received calcium salts had significantly decreased calcium concentrations with the 15 mM NH_4^+ treatment. This may indicate that ammonium did not compete with calcium aquisition in radishes when calcium in the soil was high but did so in spruce seedlings in unamended soil.

The magnesium concentrations in indicator plants and red spruce seedlings were not affected as expected and reported by previous workers. Beans and tomatoes had decreased calcium and magnesium concentrations and dry weights due to NH_4^+ treatments in nutrient solution (Maynard and Barker, 1969; Torres de Claassen and Wilcox, 1974). Mulder (1956) concluded that NH_4^+ nutrition increases the magnesium requirements of plants. Evers (1964) treated Norway spruce with NO_3^- and NH_4^+ alone and in combination in growth media with pH 8, 6, and 3.3. He reported lower dry weight and lower calcium and magnesium accumulation with NH_4^+ nutrition and soil acidity of below pH 6. Those negative NH_4^+ effects were eliminated in Evers' study, as calcium was supplied, though the dry weights were still lower than those with NO_3^- nutrition. Significant calcium and magnesium depressions in Norway spruce due to increased NH_4^+ nutrition were reported by Ingestad (1979). Boxman and Roelofs (1988) detected over an observation period of 4 hours a rapid loss of potassium, calcium, and magnesium from Scots pine roots incubated in a solution containing 0.09 mM NO_3^- and 1 mM NH_4^+.

Despite statistically measurable differences, actual nitrogen concentrations in the chlorotic spruce seedlings treated with 11.25 and 15 mM NH_4^+ were only 1 to 5 mg g^{-1} lower in a total concentration of about 20 mg g^{-1} than those with treatments containing a higher ratio of NO_3^-. The nitrogen present in the chlorotic plant organs was to some extent a product of protein decomposition. Maynard and Barker (1969) reported that in chlorotic tissue, nitrogen may be present as free amino acids, amides, and NH_3.

From the experiments in Chapter IV, it was shown that soil acidity and ammonium did not restrict the accumulation of potassium, calcium, and magnesium in indicator plants. Ammonium does not affect potassium accumulation (Barker et al., 1988). Calcium and magnesium were supplied by the limestone or other amendments. The dry weights of the needles and radish plants were decreased in the mineral horizon with NH_4^+ treatments. Ammonium most likely restricted accumulation of plant matter long before the chlorosis was visible. Despite optimum mineral element concentrations in the spruce seedlings in the present study there must have been a masked elemental deficiency on the tissue level that ultimately gave way to ammonium toxicity. It has to be kept in mind that the accumulation of mineral elements will be lower in plants with low dry weights than of plants with higher dry weights and the same mineral element concentrations. Ingestad and Kähr (1985) reported that growth of Norway spruce was strongly enhanced with increasing amounts of nitrogen fertilization and detected a simultaneous decrease of K, P, Ca, and Mg due to a dilution effect, that is, increased growth with a corresponding increase in nutrient accumulation.

New growth of seedlings depends largely on the nutrition in the last growing period in which the buds get formed (Leyton, 1958). In the present study, potassium and magnesium were probably present in the buds and were translocated from the older needles and the stems as growth started. Calcium that was affected by the treatments needs to be absorbed newly. Like no other element, calcium absorption is strongly dependent on growing fine roots, for 80 % of calcium in seedlings is taken up by root tips (Marschner et al., 1991). Due to the dependency on mass flow of calcium in shoots, fast-growing tissues and organs with low transpiration will suffer calcium deficiencies Kirkby and Pilbeam, 1984). Magnesium and potassium deficiencies are likely once the supply from the soil is depleated and the reserves within the plant are exhausted. The work of Mitchell (1934; 1939) with red spruce seedlings and Zöttl and Kennel (1963) with mature Norway spruce demonstrated that ammonium application increased the dry weight of needles and wood growth of trees as long as no other plant nutrient was limiting.

Soil from the organic horizon appeared to promote more plant growth than soil from the a horizon. In the forest, trees spread their fine roots to a large proportion in the organic horizon and at the interface between O and A horizon. Leyton (1958) attributed the large root mass located in the organic horizon to better aeration, higher nitrogen concentrations, and a root growth stimulating effect from the organic compounds. Kraske and Fernandez (1990) tested O and B horizons from spodosols on conifer seedling growth. They observed seven times higher needle weights and a doubling in nitrogen concentrations where conifers were grown in organic horizons.

Trees in the pure red spruce stand from where the soil samples were taken appeared to be healthier than red spruce that grew in a mixture with hardwood species 200 m away. The declining red spruce in Becket (see Chapter III) grew also a mixed forest stand. Deciduous trees may be more competitious in mineral element assimilation so that conifers, deprived of nutrients will decline first.

In the present experiment, spruce seedlings were treated with the same nutrient solution composition (N, P, K, Ca, and Mg at 15, 1, 6, 5, 2 mM, respectively) as used for indicator plants but at lower daily rates. Salt accumulated visibly in the pots and had to be flushed periodically to prevent salt injury. Leyton (1954) and Mitchell (1939) used nutrient solutions similar to Hoagland's solution in soil-grown red spruce. Day and

Robbins (1950) applied 7 mM N, 0.5 mM P, 3 mM K, 2.5 mM Ca, and 1 mM Mg in sand culture to red spruce seedlings, whereas Marschner et al. (1991) used only one-tenth of the latter to study mineral element uptake of roots of Norway spruce in solution culture. Since the seedlings in the present study grew healthy in the treatment containing nitrate it can be concluded that accumulating salt concentrations did not harm the plant roots.

This study concluded that ammonium was beneficial for plant growth as long as sufficient calcium and magnesium was supplied. Deficiencies of either element led to adverse effects with increasing ammonium. It was not possible to study calcium deficiency induced by ammonium with radishes because they would not grow in the forest soil without $CaCO_3$ amendment. The ammonium treatments did not induce calcium and magnesium deficiencies if calcium and magnesium were supplied with the nutrient solutions. The cause of the restriction of plant growth due to the NH_4^+ treatment and the mineral horizon cannot be attributed to a single factor. A complex syndrome of low nutrition, acid soils, and input of nitrogen from precipitation may contribute to forest decline. Further studies about mineral nutrition in red spruce seedlings may evaluate the effect of a mild ammonium and mineral element stress for more than one growing season to assess exhaustion of the reserves in the seedlings.

CHAPTER VI

CONCLUSIONS

From file studies of mature trees in western Massachusetts evidence was found that declining red spruce suffering needle loss were P, Ca and Mg deficient and chlorotic balsam fir were K and Mg deficient in relation to standards proposed in the literature. Declining or healthy red spruce trees had low N and K concentrations in the foliage. The cause for the needle loss in declining trees was probably due to Mg deficiency. Chlorotic balsam fir contained sufficient nitrogen. The mineral element deficiencies were most likely due to low mineral element concentrations in the soil. Soil analysis showed low K, Ca, and Mg concentrations in the mineral soil. Elements were concentrated in the organic horizon.

Greenhouse experiments with indicator plants and red spruce showed that NH_4^+ is a potential cause for growth restriction and calcium deficiency in plants. Ammonium was utilized by radishes in acid soil ($pH < 4.5$) when liming and $MgSO_4$ were provided. Growth of red spruce seedlings in acid soil was decreased when NH_4^+ supplied in a nutrient solution exceeded 25 % of the nitrogen applied. The treatments in the present study did not restrict the accumulation of Ca or Mg to levels of deficiency in terms of elemental concentrations present in the tissue. It appears that there was no competition between NH_4^+ and calcium or magnesium upon uptake by the roots in radishes but perhaps for calcium in red spruce seedlings. Calcium and magnesium deficiency symptoms occurred in radishes and red spruce even though calcium and magnesium levels were not below accepted standards. The deficienciy symptoms or ammonium toxicity symptoms observed were apparently due to the effect that NH_4^+ increased the requirement for Ca and Mg or suppressed the utilization by plants. Input of NH_4^+ from acid rain might lead to Ca and Mg deficiencies in trees grown in marginal forest soils.

BIBLIOGRAPHY

Alexander, M. 1965. Nitrification. p. 307-333. In: W.V. Bartholomew and F.E. Clark (eds.), Soil nitrogen. American Society of Agronomy, Madison, WI.

Allinger, P., G. Michael, and P. Martin. 1969. Einfluss von Cytokininen und anderen Wuchsstoffen auf die Stoffverlagerung in abgeschnittenen Blättern. Flora (Jena) Abt. A. 160:538-551.

Arnon, D.I., W.E. Fratzke, and C.M. Johnson. 1942. Hydrogen ion concentration in relation to absorption of inorganic nutrients by higher plants. Plant Physiol. 17:515-524.

Arnon, D.I., and C.M. Johnson. 1942. Influence of hydrogen ion concentration on growth of higher plants under controlled conditions. Plant Physiol. 17:525-539.

Barker, A.V., J.F. Laplante, and R.A. Damon. 1983. Growth and composition of radish under various regimes of nitrogen nutrition. J. Am. Soc. Hort. Sci. 108:1035-1040.

Barker, A.V., Ready, K., and H.A. Mills. 1988. Critical calcium concentrations in radish grown under various regimes of nitrogen nutrition. Plant Nutrition 11(12):1727-1738.

Barker, A.V., R.J. Volk, and W.A. Jackson. 1966. Root environment acidity as a regulatory factor in ammonium assimilation by the bean plant. Plant Physiol. 41:1193-1199.

Basynski, T., M. Warcholowa, M. Krupa, Z. Tukendorf, A. Krol, and D. Wolinska. 1980. The effect of magnesium deficiency on photochemical activities of rape and buckwheat chloroplasts. Z. Pflanzenphysiol. 99:295-303.

Baule, H., and C. Fricker. 1967. Die Düngung von Waldbäumen. Bayrischer Landwirtschafts Verlag, München.

Beyschlag, W., M. Wedler, O.L. Lange, and U. Heber. 1987. Einfluss einer Magnesiumdüngung auf Photosynthese und Transpiration von Fichten an einem Magnesium-Mangelstandort im Fichtelgebirge. Allgemeine Forst Zeitschrift 27/28/29:738-741.

Boxman, A.W., and J.G.M. Roelofs. 1988. Some effects of nitrate versus ammonium nutrition on the nutrient fluxes in Pinus silvestris seedlings. Effects of mycorrhizal infection. Can. J. Bot. 66:1091-1097.

Bremner, J. 1965. Total nitrogen. p. 1149-1341. In: C.A. Black (ed.), Methods of soil analysis. Agronomy 9. American Society of Agronomy, Madison, WI.

Bruck, R.I. 1985. Boreal montane ecosystem decline in the southern appalachian mountains: potential role of anthropogenic pollution. p. 137-155. Symposium on the Effect of air pollutants on forest ecosystems. Univ. of Minnesota, St. Paul, MN.

Buol, S.W., E.D. Hole, and R.J. McCracken. 1980. Soil genesis and classification. 2nd edition. Iowa State University Press. Ames, IA.

Burström, H.G. 1968. Calcium and plant growth. Biol. Rev. 43:287-316.

Cataldo, D.A., M. Haroon, L.E. Schrader, and V.L. Young. 1975. Rapid colorometric determination of nitrate in plant tissue by nitration of salicylic acid. Commun. Soil Sci. and Plant Anal. 6(1):71-80.

Coutts, M.P. and J.J. Philipson. 1980. Mineral nutrition and tree root growth. p. 123. In: D. Atkinson, J.E. Jackson, R.O. Sharples, and W.M. Waller (eds.), Mineral nutrition of fruit trees. Butterworth, Boston.

Day, G.M., and W.R. Robbins. 1950. Observations on the growth of red spruce in sand culture. J. For. 48:689-692.

Dethier, D.P., Jones, S.B., Feist, T.P., and Ricker, J.E. 1988. Relations among sulfate, aluminum, iron, dissolved organic carbon, and pH in upland forest soils of northwestern Massachusetts. Soil. Sci. Soc. Am. J. 52:506-512.

Eaton, F.M. 1966. Chlorine. p. 98-135. In: H.D. Chapman (ed.), Diagnostic criteria for plants and soils. Univ. of California, Division of Agricultural Sciences, Riverside.

Edfast, A.-B., T. Naesholm, and A. Ericson. 1990. Free amino acid concentrations in needles of Norway spruce and Scots pine trees on different sites in areas with two levels of nitrogen deposition. Can. J. For. Res. 20: 1132-1136.

English, J.E. and D.N. Maynard. 1978. A key to nutrient disorders of vegetable plants. HortScience 13:28-29.

Evers, F.H. 1964. Die Bedeutung der Stickstofform für Wachstum und Ernährung der Pflanzen insbesondere der Waldbäume. Mitteilungen des Vereins für forstliche Standortkunde und Forstpflanzenzüchtung 14:19-37.

Federer, C.A. 1983. Nitrogen mineralization and nitrification: depth variation in four New England forest soils. Soil Sci. Soc. Am. J. 47:1008-1014.

Federer, A.C., and J.W. Hornbeck. 1985. The buffer capacity of forest soils in New England. Water Air Soil Pollut. 26:163-173.

Forschner, W., and A. Wild. 1988. Comperative investigation on the nutrient composition of healthy and injured spruce of different locations. p. 604-608. In: Mathy P. (ed.), Air Pollution and Ecosystems. Proceedings of a Commission of the European Community Symposium, Grenoble, France, May 1988. D. Reidel Publishing.

Foy, C.D. 1984. Physiological effects of hydrogen, aluminum, and manganese toxicities in acid soil. p. 57-97. In: R.W. Pearson and F. Adams (eds.), Soil acidity and liming, 2nd edition Agronomy 12. American Society of Agronomy, Madison, WI.

Foy, C.D., R.L. Chaney, and M.C. White. 1978. The physiology of metal toxicity in plants. Annu. Rev. Plant Physiol. 29:511-566.

Friedland, A.J., G.J. Hawley, and R.A. Gregory. 1988. Red spruce (Picea rubens Sarg.) foliar chemistry in northern Vermont and New York, U.S.A. Plant Soil 105: 188-193.

Garten, C.T.Jr., and P. J. Hanson. 1990. Foliar retention of ^{15}N- Nitrate and ^{15}N-Ammonium by red maple (Acer rubrum) and white oak (Quercus alba) leaves from simulated acid rain. Envir. Exp. Bot. 30(3):333-342.

Gijsman, A.J. 1990a. Rhizosphere pH along different root zones of Douglas-fir (Pseudotsuga menziesii), as affected by source of nitrogen. Plant and Soil 124: 161-167.

Gijsman, A.J. 1990b. Nitrogen nutrition of Douglas-fir (Pseudotsuga menziesii) on strongly acid sandy soil. I. Growth, nutrient uptake and ionic balance. Plant Soil 126:53-61.

Gijsman, A.J. 1990c. Nitrogen nutrition of Douglas-fir (Pseudotsuga menziesii) on strongly acid sandy soil. II. Proton excretion and rhizosphere pH. Plant Soil 126:63-70.

Hambruckers, A., and J. Remacle. 1991. Nutritional status of declining spruce (Picea abies (L.) Karst.): Effect of soil organic matter turnover rate. Water Air and Soil Pollut. 59:95-106.

Hoagland, D.R., and D.I. Arnon. 1950. The water culture method for growing plants without soil. California Agric. Exp. Sta. Circ. 347.

Holden, M. 1965. Chemistry and biochemistry of plant pigments. Academic Press, New York. p. 461-467.

Huntington, T.G., D.R. Peart, J. Hornig, D.F. Ryan, and S. Russo-Savage. 1990. Relationships between soil chemistry, foliar chemistry, and condition of red spruce at Mount Moosilauke, New Hampshire. Can. J. For. Res. 20:1219-1227.

Ilgen, G, and Nebe, W. 1989. Jahresringchronologische Differenzierung chemischer Elemente im Holz älterer Fichten. Biol. Rundschau 27:237-247.

Ingestad, T. 1979. Mineral nutrient requirements of Pinus silvestris and Picea abies seedlings. Physiol. Plant. 45:373-380.

Ingestad, T., and M. Kähr. 1985. Nutrition and growth if coniferous seedlings at varied relative nitrogen addition rate. Physiol. Plant. 65:109-116.

Jackson, W.A. 1967. Physiological effects of soil acidification. p. 43-124. In: R.W. Pearson and F. Adams (eds.), Soil acidity and liming. Agronomy Publ. No. 12, 2nd edition. American Society of Agronomy, Madison, WI.

Johnson, A.H., T.G. Siccama, W.L. Silver, and J.J. Battles. 1989. Decline of red spruce in high-elevation forests of New York and New England. p. 85-112. In: D.C Adriano and M Havas (eds.), Acid precipitation 1. Case studies. Springer-Verlag, New York.

Kandler, O., Miller, W., and Ostner, R. 1987. Dynamik der "akuten Vergilbung" der Fichte. Epidemiologische und physiologische Befunde. Allg. Forstzeitschrift. 27/28/29:715-723.

Kaupenjohann, M., W. Zech, R. Hantschel, and R. Horn. 1987. Ergebnisse von Düngungsversuchen mit Magnesium an vermulich immissionsgeschädigten Fichten (Picea abies [L.] Karst.) im Fichtelgebirge. Forstw. Cbl. 106:78-84.

Kirkby, E.A. and D.J. Pilbeam. 1984. Calcium as a plant nutrient. Plant Cell Environ. 7:397-405.

Köstner, B., O.L. Lange, and F.C. Czygan. 1989. Jahresverlauf der Chloroplastenpigmente von Fichten in einem Waldstandort im Fichtelgebirge in Abhängigkeit von Alter und Mineralstoffgehalt der Nadeln. p. 107-118. In: Projektgruppe Bayern zur Erforschung der Wirkung von Umweltstoffen (PBWU)(ed.). Berichte der Gesellschaft für Strahlen- und Umweltforschung No 6, München.

Kraske, C.R., and I.J. Fernandez. 1990. Conifer seedling growth response to soil type and selected nitrogen availability indices. Soil Sci. Soc. Am. J. 54:246-251.

Krivian, V., and G. Schaldach. 1986. Untersuchungen zur Probenahme und -vorbehandlung von Baumnadeln zur Elementanalyse. Fresenius Z. Anal. Chem. 324:158-167.

Krogmann, D.W., A.T. Jagendorf, and M. Arnon. 1959. Uncouplers of spinach chloroplast photosynthetic phosphorylation. Plant Physiol. 34:272-277.

Krug, E.C., and C.R. Frink. 1983. Acid rain on acid soil: a new perspective. Science 221:520-525.

Kuylenstierna, J.C.I., and M.J. Chadwick. 1991. Increase in north-west Wales between 1957 and 1990. AMBIO 20(3-4): 118-119.

Lange, O.L., U. Heber, E.-D. Schulze, and H. Ziegler. 1989. Atmospheric pollutants and plant metabolism. In Schulze, E.D., O.L. Lange, and R. Oren (eds.). Forest decline and air pollution. A study of spruce (Picea abies) on acid soils. Springer-Verlag, New York.

LeBlanc, D.C. 1990. Red spruce decline on Whiteface Mountain, New York. I. Relationships with elevation, tree age, and competition. Can. J. For. Res. 20:1408-1414.

Leisen, E., M. Häussling, and H. Marschner. 1990. Einfluss von Stickstoff-Form und Konzentration und saurer Benebelung auf pH-Veränderungen in der Rhizosphäre von Fichten (Picea abies [L.] Karst.). Forstw. Cbl. 109: 275-286.

Leisen, E., und H. Marschner. 1990. Wirkung von Düngung und saurer Benebelung auf Photosyntheserate, Kohlenhydratgehalt in Nadeln und Wurzeln sowie Spross- und Wurzelwachstum von Fichten (Picea abies [L.] Karst.). Forstw. Cbl. 109:264-274.

Leyton, L. 1954. The growth and mineral nutrition of spruce and pine in heathland plantations. Inst. Pap. Imp. For. Inst., Oxford No. 31.

Leyton, L. 1958. Some aspects of forestry research in Great Britain. Univ. of Toronto Forestry Bull. No.5.

Likens, G.E., F.H. Bormann, R.S. Pierce, J.S. Eaton, and N.M. Johnson. 1977. Biogeochemistry of a forested ecosystem. Springer-Verlag, New York.

Lovett, G.M., W.A. Reiners, and R.K. Olson. 1982. Cloud droplet deposition in subalpine balsam fir forests: hydrological and chemical inputs. Science 218:1303-1304.

McColl, J.M. and M.K. Firestone. 1987. Cumulative efects of simulated acid rain on soil chemical and microbial characteristics and conifer seedling growth. Soil Sci. Soc. Am. J. 51:794-800.

MacDonald, N.W., J.B. Hart, Jr., and P.V. Nguyen. 1986. Simulated acid rain effects on jack pine seedlings establishment and nutrition. Soil Sci. Soc. Am. J. 50: 219-225.

McElhannon, W.S., and H.A. Mills. 1978. Influence of percent NO_3^-/NH_4^+ on growth, N absorption, and assimilation by Lima beans in solution culture. Agronomy J. 70:1027-1032.

Marschner, H. 1986. Mineral nutrition in higher plants. Academic Press, London. p. 484-497.

Marschner, H., Häussling, M., and E. George. 1991. Ammonium and nitrate uptake rates and rhizosphere pH in non-mycorrhizal roots of Norway spruce [Picea abies (L.) Karst.]. Trees 5:14-21.

Matzner, E. 1989. Acidic precipitation: case study Solling. p. 39-83. In: D.C Adriano and M. Havas (eds.), Acidic precipitation Volume 1: Case studies. Springer-Verlag, New York.

Maynard, D.N., and A.V. Barker. 1969. Studies on the tolerance of plants to ammonium nutrition. J. Am. Soc. Hort. Sci. 94:235-239.

Mengel, K., M. Th. Breininger, and H.J. Lutz. 1988. Effect of simulated acidic fog on carbohydrate leaching, CO_2 assimilation and development of damage symptoms in young spruce trees (Picea abies L. Karst). Envir. Exp. Bot. 30(2):165-173.

Mitchell, H.L. 1934. Pot culture tests of forest soil fertility. Black Rock Forest Bull. No 5.

Mitchell, H.L. 1939. The growth and nutrition of white pine (Pinus strobus L.) seedlings in cultures with varying nitrogen, phosphorus, potassium and calcium. Black Rock Forest Bull. No.11.

Mollitor, A.V., and D.J. Raynal. 1982. Acid precipitation and ionic movement in Adirondac forest soils. Soil Sci. Soc. Am. J. 46:137-141.

Mulder, E.G. 1956. Nitrogen-magnesium relationships in crop plants. Plant Soil 7(4):341-376.

Nebe, W. 1991. Veränderungen der Stickstoffversorgung- und Magnesiumversorgung immisionsbelasteter älterer Fichtenbestände in ostdeutschen Mittelgebirgen. Forstw. Cbl. 110:4-12.

Nihlgard, B. 1985. The ammonium hypothesis -an additional explanation to the forest dieback in Europe. AMBIO 14(1):2-8.

Oren, R., E.-D. Schulze, K.S. Werk, and J. Meyer. 1988. Performance of two Picea abies (L.) Karst. stands at different stages of decline. Oecologia 77:163-173.

Rich, I. 1965. Element analysis by flame photometry. p. 849-865. In: C.A. Black (ed.), Methods of soil analysis. Agronomy 9. American Society of Agronomy, Madison, WI.

Riha, SJ., B.R. James, G.P. Senesac, and E. Pallant. 1986. Spatial variability of soil pH and organic matter in forest plantations. Soil Sci. Soc. Am. J. 50:1347-1352.

Roelofs, J.G.M., A.J. Kempers, A.L.F.M. Houdjik, and J. Jansen. 1985. The effect of air-borne ammonium sulphate on Pinus nigra var. maritima in the Netherlands. Plant Soil 84:45-56.

Rollwagen, B.A., and R.J. Zasoski. 1988. Nitrogen source effects on rhizosphere pH and nutrient accumulation by pacific northwest conifers. Plant Soil 105:79-86.

Scanu, R.J. 1988. Soil Survey of Berkshire County, Massachusetts. USDA, Soil Conservation Service.

Schenk, M.K., and J. Wehrmann. 1979. The influence of ammonium in nutrient solution on growth and metabolism of cucumber plants. Plant Soil 52:403-414.

Schulze, E.-D., Oren, R.,Zimmermann, R. 1987. Die Wirkungen von Immissionen auf 30-järhrige Fichten in mittleren Höhenlagen des Fichtelgebirges auf Phyllit. Allg. Forstzeitschrift. 27/28/29:725-730.

Schulze, E.-D. 1989. Air pollution and forest decline in a spruce (Picea abies) forest. Science 244:776-783.

Scott, J.T., T.G. Siccama, A.H. Johnson, and A.R. Breisch. 1984. Decline of red spruce in the Adirondacks, New York. Bull. Torrey Bot. Club 111:438-444.

Senser, M., and K.-A. Höpker. 1989. Auftreten und Erholung der "akuten Vergilbung" bei Fichte durch unterschiedliche Nährstoffversorgung. p. 61-75. In: Projektgruppe Bayern zur Erforschung der Wirkung von Umweltstoffen (PBWU)(ed.). Berichte der Gesellschaft für Strahlen- und Umweltforschung No 6, München.

Siccama, T.G., M. Bliss, and H.W. Vogelmann. 1982. Decline of red spruce in the Green Mountains of Vermont. Bull. Torrey Bot. Club 109:162-168.

Soil Survey Staff. 1990. Keys to soil taxonomy. Agency for international development, USDA, Soil Management Support Services. Technical Monograph No. 19, fourth edition. Virginia Polytechnic Institute and State University.

Street. H.E., and D.E.G. Sheat. 1958. The absorption and availability of nitrate and ammonia. p. 150-165. In: W. Ruhland (ed.), Der Stickstoffumsatz Handbuch der Pflanzenphysiologie Vol. 8. Springer-Verlag.

Süchting, H. 1949. Lehrbuch der Bodenkunde und Pflanzenernährung. Landbuch Verlag, Hannover.

Tesche, M. 1991. Stress und Decline bei Waldbäumen. Forstw. Cbl. 110:56-65.

Tjepkema, J. 1979. Nitrogen fixation in forests of central Massachusetts. Can. J. Bot. 57:11-16.

Tjepkema, J.D., and R.J. Cartica. 1981. Atmospheric concentration of ammonia in Massachusetts and deposition on vegetation. Nature (London) 294:445-446.

Torres de Claassen, M.E., and G.E. Wilcox. 1974. Effect of nitrogen form on growth and composition of tomato and pea tissue. J. Am. Soc. Hort. Sci. 99:171-174.

Ulrich, B. 1990. Waldsterben: Forest decline in west Germany. Environ. Sci. Technol. 24(4):436-441.

Van Breemen, N., C.T. Driscoll, and J. Mulder. 1984. Acidic deposition and internal proton sources in acidification of soils and waters. Nature (London) 307:599-604.

Van Diest, A. 1989. Eintrag von NO_3^- und NH_4^+ in niederländische Waldbestände und deren Auswirkungen auf die N-Ernährung und den Vitalitätszustand. Kali-Briefe (Buentehof) 19:391-401.

Van Miegroet, H., and D.W. Cole. 1985. Acidification sources in red alder and Douglas fir soils - Importance of nitrification. Soil Sci. Soc. Am. J. 49:1274-1279.

Veneman, P.L.M, and S.M. Bodine. 1982. Chemical and morphological soil characteristics in a New England drainage toposequence. Soil Sci. Soc. Am. J. 46:357-363.

Watanabe, F.S., and S.R. Olsen. 1965. Test of ascorbic acid method for determining phosphorous in water and $NaHCO_3$ extracts from soil. Soil Sci. Soc. Am. Proceedings 29(6):677-678.

Wittich, W. 1959. Bodenkundliche und pflanzenphysiologische Grundlagen der Kalkdüngung im Walde. Der Wald braucht Kalk, Kölner Universitätsverlag.

Zech, W. 1973. Besonderheiten im Ernährungszustand chlorotischer Fichten auf kalkreichem Boden. Forstw. Cbl. 1-9.

Zech, W., and E. Popp. 1983. Magnesiummangel, einer der Gründe für das Fichten- und Tannensterben in NO-Bayern. Forstw. Cbl. 102:50-55.

Zöttl, H., and R. Kennel. 1963. Nutrition and growth of mature spruce stand after fertilizing with gaseous NH_3 and N-salts. Forstw. Cbl. 82:76-100.

Zöttl, H.W. 1990. Ernährung und Düngung der Fichte. Forstw. Cbl. 109:130-137.